BOOK OF ABSTRACTS

FEBS Special Meeting: Expanding frontiers in AARS research

Sep 28th - Oct 3rd 2025 Dubrovnik, Croatia

FEBS Special Meeting
Expanding frontiers in aminoacyl-tRNA synthetase research

Organized by:
University of Zagreb Faculty of Science
Croatian Society of Biochemistry and Molecular Biology

September 28th - October 3rd 2025
Dubrovnik, Croatia

Book of Abstracts

Book of Abstracts of the FEBS Special Meeting: Expanding frontiers in aminoacyl-tRNA synthetase research

Editors
Ita Gruic-Sovulj, Lluís Ribas de Pouplana, Morana Dulic, Jasmina Rokov-Plavec

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Lluís Ribas de Pouplana	ICREA/IRB Barcelona

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Welcome address

Dear participants of AARS2025,

We are pleased to welcome you to the FEBS Special Meeting “Expanding Frontiers in Aminoacyl-tRNA Synthetase Research.” This is the first AARS meeting to take place in Croatia despite its long history of tRNA and AARS research.

This meeting aims to strengthen the links between AARS research and nearby communities by featuring keynote and invited talks of leading scientists from closely related fields.

We have put together a diverse and engaging scientific program. We have five keynote lectures given by world-renowned scientists, including the EMBO keynote lecture by Nenad Ban, the IUBMB keynote lecture by Hiroaki Suga and the Women in Science keynote lecture by Rita Horvath. The program also features an Opening talk by Paul Schimmel, a Closing talk by Paul Fox, 29 invited and 28 selected talks. We will also hold four poster sessions and 19 speed talks by poster authors.

To promote cross-field interactions among basic scientists, clinicians, the biotech industry, and patients affected by AARS-related disorders, we have planned a Patient-oriented session, that will include a community round table and one-on-one meetings designed to foster meaningful and interactive exchange among all stakeholders.

We are excited to introduce you to Croatian culture. Our social program includes a boat trip to Dubrovnik and a conference dinner that will highlight our traditional regional cuisine. We hope these events will create lasting memories and promote community building.

We are deeply grateful for the support from FEBS, our sponsors and donors. Their generosity has been instrumental in making this meeting possible.

We extend our warm thanks to all of you for joining us with an open mind to discuss science. Above all, it will be your contributions that will make this meeting inspiring and unforgettable.

Ita Gruic-Sovulj

Lluís Ribas de Pouplana

Big thank you to our partners and sponsors

Diamond sponsor

Gold sponsor

Sponsors

The organizers are particularly grateful to Paul Fox, Serpil Erzurum and an anonymous donor for their generous support.

Conference history

2025	14th International Symposium on AARSs FEBS Special meeting: Expanding frontiers in aminoacyl-tRNA synthetase research, **Dubrovnik, Croatia**
2023	13th International Symposium on AARSs IUBMB Focused Meeting, **Grand Bend, Canada**
2019	12th International Symposium on AARSs, **Hangzhou, China**
2017	11th International Symposium on AARSs IUBMB Focused Meeting, **Clearwater, USA**
2015	10th International Symposium on AARSs, **Barcelona, Catalonia, Spain**
2013	9th International Symposium on AARSs, **Hakone, Japan**
2011	8th International Symposium on AARSs, **Salt Lake City, USA**
2008	7th International Symposium on AARSs International Conference on AARSs: From basic mechanism to system biology, **Veyrier du Lac, France**
2006	6th International Symposium on AARSs 2006 International Conference on AARSs: From the Genetic Code to Human Disease & Medicine, **San Diego, USA**
2004	5th International Symposium on AARSs 2004 International Conference on AARSs: Ancient Molecular for Future Biology and Medicine, **Seoul, South Korea**
2002	4th International Symposium on AARSs Asilomar Conference on AARSs in Biology, Medicine & Evolution, **Asilomar, USA**

1998	3rd International Symposium on AARSs EMBO Workshop on Structure and Function of AARSs, **Mittelwihr, France**
1997	2nd International Symposium on AARSs Keystone Symposium on AARS sin Biology & Disease, **Taos, USA**
1990	1st International Symposium on AARSs NATO Workshop on AARSs, **Autrans, France**

Information

Registration

Registration will take place at the registration desk in front of the congress center of the Sheraton Dubrovnik Riviera Hotel from 14:00 on Sunday, September 28th.

Registration fee for participants includes admission to all scientific program sessions, Book of Abstracts, congress materials, accommodation, four lunches, three regular dinners and refreshments as set in the program, welcome dinner and reception, excursion and conference dinner. Registration fee for accompanying persons includes four lunches, three regular dinners, welcome dinner and reception, excursion and conference dinner.

We kindly ask you to complete the online survey at the end of the meeting. The certificate of attendance can be downloaded from the official website upon completion of survey.

Language

The official language of the conference is English.

Lectures and oral presentations

The speakers are kindly asked to deliver their presentations (USB stick) in advance to our technician (for morning session by the end of evening session previous day; for afternoon session by the end of the morning session the same day). The use of personal laptops is not possible due to potential incompatibilities and timing issues.

All lectures will take place in the Orlando C hall.

Poster presentations

Poster presentations will take place in the adjacent congress rooms (Lokrum and Lovrjenac). Posters should be mounted by Monday 29th September by 9:00 and removed on Thursday until 19:00. The presenting authors are expected to be next to their poster according to the schedule and to the List of posters in the Book of Abstracts (Odd numbers: Monday 10:25-11:25 and Wednesday 20:30-22:00; Even numbers: Monday 20:30-22:00 and Tuesday 10:25-11:25).

Poster panels and mounting accessories will be available at site.

All posters will be evaluated by the Scientific board. Poster Award will be given to the best presenters at the closing ceremony.

Social events

Sunday, September 28th	19:00	Welcome dinner and drinks (Sheraton Dubrovnik Riviera Hotel)
Tuesday, September 30th	14:30	Excursion*
Tuesday, September 30th	20:00	Conference dinner (Sheraton Dubrovnik Riviera Hotel)

* Excursion will start with the gathering of the participants in front of the Sheraton Dubrovnik Riviera hotel main entrance. We will leave the meeting point toward the excursion boat at 14:30. No additional transportation will be available after 14:30 to join the Excursion.

Program

Sunday, 28th September

Opening ceremony		
17:00		**Ita Gruic-Sovulj and Lluís Ribas de Pouplana** Welcome address
17:20		**Zrinka Kovarik (FEBS)** Introduction to Federation of European Biochemical Societies (FEBS)
Chair: Susan Martinis		
17:30	IT1	**Opening lecture** **Paul Schimmel** Boolean analysis of human tRNA synthetase splice variants
Chair: Jasmina Rokov Plavec		
18:00	KL1	**EMBO Keynote lecture** **Nenad Ban** Ribosome upside down: co-translational protein biogenesis at the tunnel exit
19:00	Welcome dinner and reception	

Monday, 29th September

Chair: Ita Gruic-Sovulj

08:30	KL2	**Michal Sharon** Expanding the proteasome paradigm: uncovering the independent roles of the 20S core complex

Canonical Biochemistry 1
Chair: Rebecca Alexander

09:15	IT2	**Karin Musier-Forsyth** Surprising enzymatic activity of OB-fold containing multi-aminoacyl-tRNA synthetase adaptor proteins
09:35	ST1	**Shobha Kruparani Polarumi** Chiral discrimination and metal ion mediated translation quality control in alanyl-tRNA synthetase
09:50	ST2	**Guillaume Hoffmann** Decoding zinc domain dynamics and function in leucyl-tRNA synthetase
10:05	IT3	**Osamu Nureki** Structural basis for tRNA-dependent lipid aminoacylation
10:25		**Poster session (Odd numbers) and coffee break**

Canonical Biochemistry 2
Chair: Xiang-Lei Yang

11:25	IT4	**Ita Gruic-Sovulj** Is speed the key? Exploring divergence in bacterial $tRNA^{Ile}$ recognition
11:45	IT5	**Rajan Sankaranarayanan** Chiral proofreaders, their variants and their functional roles

12:05	ST3	**Yinuo Wang** An openly available toolbox for the study of tRNA synthetases
12:20	ST4	**Oscar Vargas-Rodriguez** Coexisting tryptophanyl-tRNA synthetases endow a human microbial pathogen with distinct fitness advantages
12:35		**Poster speed talks**
		Luciano Dolce (P7) Structural basis for leucyl-tRNA synthetase assembly into the human multi-tRNA synthetase complex by isoleucyl-tRNA synthetase
		Petra Kozulic (P16) Exploring molecular adaptations of tRNA recognition in bacterial isoleucyl-tRNA synthetase type 2
		Laura Ruduša (P31) Bacterial seryl-tRNA synthetases as therapeutic targets: a fragment-based approach to inhibition
		You Wu (P38) tRNA-derived SINE Repetitive elements regulate porcine embryonic genome activation
		Stanley Xie (P39) Inducing bacteria to self-destruct by hijacking the tRNA-charging process
		Ryosuke Zaitsu (P40) Development of cyclic peptide inhibitors targeting system Xc via aminoacyl-tRNA synthetase–mediated synthesis and structural characterization by cryo-EM
13:00	Lunch	

Evolution
Chair: Joe Chihade

15:00	IT6	**Klara Hlouchova** Pre-LUCA protein evolution
15:20	ST5	**Jordan Douglas** Phylogenetic reconstruction of ancestral coding alphabets using aaRS sequences
15:35	ST6	**Joshua Maiklem** A catalytic pathway to support prebiotic linkage between amino acids and RNA
15:50	ST7	**Charlie Carter** Structural modularity, functional information, and the genesis of genetic coding
16:05	ST8	**Aleksandar Radakovic** Historical reconstruction of the glutamate-to-glutamine specificity switch in a resurrected ancient aminoacyl-tRNA synthetase
16:20		**Poster speed talks**
		Elisabeth Gardiner (P9) The use of therapeutic suppressor tRNAs for Duchenne muscular dystrophy and dilated cardiomyopathy
		Hanako Murayama (P26) Architectural basis of tRNA-dependent sterol aminoacylation
		Gregor Spruk (P35) The use of genetic code expansion in the production of highly stable protein nanopores
		Ekaterina Zakharova (P41) Expanding the genetic and clinical spectrum of neurological disorders associated with tRNA synthetase deficiencies in Russian patients

		Yunfang Zhang (P42) Deciphering tRNA repertoires and translation coordination during mouse early embryogenesis by ORACLE-tRNAseq
16:40	Coffee break	
Fidelity *Chair: Rajan Sankaranarayanan*		
17:10	IT7	**Lorenzo Leiva Araya** Homocysteine thiolactone from translation proofreading is an endogenous ligand for cell-cell signaling by the receptor SdiA
17:30	IT8	**Jiqiang Ling** Translational fidelity and the integrated stress response in eukaryotes
17:50	ST9	**Marko Mocibob** Proteomic profiling of isoleucine mistranslation in *Escherichia coli*
18:05	ST10	**Arshia Arasteh Kani** Drive genetic code expansion with a dual fluorescent reporter and FACS-based selection of functional aminoacyl-tRNA synthetases
18:20	ST11	**Natalie Krahn** Engineering translation machinery for production of post-translationally modified selenoproteins in *E. coli*
18:35		**Poster speed talks**
		Francesca Brevi (P3) Translation perturbance in tRNA synthetase-defective trichothiodystrophy
		Mirim Jin (P13)

	Targeting highly secreted tryptophanyl-tRNA synthetase: a potential theranostic for hypercytokinemic severe sepsis **Stephanie Laureano Figueroa (P19)** An allelic series at R329 AARS1 reveals loss-of-function intolerance and variable dominant-negative effects **Linda Rosetti (P29)** Treatment of KARS1 deficiency with lysine supplementation in a pediatric patient: a three-year clinical update
19:00	Dinner
20:30	**Poster session with Cheese and Wine (Even numbers)** *Sponsored by Selvita*

Tuesday, 30th September

Chair: Lluis Ribas de Pouplana

08:30	KL3	**Simon Alberti** RNA condensates at the nexus of cellular stress, disease and aging

Non-canonical functions 1
Chair: Tamara Hendrickson

09:15	IT9	**Solene Zuttion** Vacuolar echoforms of yeast cytosolic aminoacyl-tRNA synthetases are modulating components of TORC1
09:35	ST12	**Igor Zivkovic** Unknown role of arginyl-tRNA synthetase paralogue with a variation in the HIGH motif
09:50	ST13	**Joongoo Lee** Expanding ribosomal catalysis via non-canonical monomers
10:05	IT10	**Xiang-Lei Yang** WHEP domain of glycyl-tRNA synthetase regulates neuropilin 1 binding and vascular permeability
10:25		**Poster session (Even numbers) and coffee break**

Non-canonical functions 1
Chair: Marko Mocibob

11:25	IT11	**Jasmina Rokov-Plavec** Role of aminoacyl-tRNA synthetases in oxidative stress response
11:45	ST14	**Jiadong Zhou** Probing secretion mechanism and topology of extracellular glycyl-tRNA synthetase

12:00	ST15	**Vasundhara Singh** The tRNA multisynthetase complex is critical for the cardiac response to stress
12:15	ST16	**Jacob Sebastian Lindqvist Muci** Investigating the scope of thioester-mediated aminoacylation of RNA with non-canonical amino acids
12:30	ST17	**Abhishek Chatterjee** Virus-assisted directed evolution of nonsense suppressor tRNAs in mammalian cells
12:45		**Poster speed talks** **Jenica Kakadia (P14)** HARS deficiency is rescued by tRNA supplementation in a humanized yeast model of Charcot-Marie-Tooth disease **Vanessa Lewandowski (P20)** Translation-mediated selective motor neuron degeneration in CMT- pathology associated with mutations in aaRS **Maria Mahmood (P23)** Investigating the underlying molecular basis of CMT-causing AlaRS pathogenic alleles by using *S. cerevisiae* as a model organism
13:00	Lunch	
14:30	Excursion	
20:00	Conference dinner	

Wednesday, 1st October

Chair: Albena Jordanova

08:30	KL4	**Women in Science Keynote Lecture** **Rita Horvath** Mitochondrial tRNA synthetase disorders: moving towards therapy

Patient-oriented session 1
Chair: Anthony Antonellis

09:15	IT12	**Marie Sissler** How the analysis of pathology-related variants shed light on fundamental process: the case of PARS2 variants
09:35	ST18	**Maria Menafra** Drosophila models of histidyl-tRNA synthetase-associated peripheral neuropathy
09:50	ST19	**Santiago Movilla** Engineered LysRS switches: uncoupling translation from Ap4A signaling with therapeutic implications
10:05	IT13	**Albena Jordanova** The nuclear function of TyrRS as a mediator of stress response in the Charcot-Marie-Tooth neuropathy
10:25	Coffee break	

Patient-oriented session 2
Chair: Haissi Cui

11:00	IT14	**Zoya Ignatova** tRNA-based therapies: roadblocks en route to their establishment as treatment for diverse pathologies
11:20	ST20	**Ilka Heinemann** tRNA and amino acid supplementation rescues mistranslation caused by pathogenic HARS alleles

11:35	**Community round table**	
13:00	**Patients meet the experts lunch**	
14:30	**Patients and scientists: Face-to-face interviews**	
Therapeutics and technology 1 *Chair: Chris Ahern*		
15:30	IT15	**Leslie Nangle** Advancing a therapeutic platform based on tRNA synthetases for treatment of fibrotic lung diseases
15:50	IT16	**Andres Palencia** Structural studies on protein–RNA complexes and applications for the development of novel therapeutics
16:10	ST21	**Jie Chen** Leucyl-tRNA synthetase as a therapeutic target for Duchenne muscular dystrophy
16:25	ST22	**Leann Tilley** Hijacking aminoacyl-tRNA synthetases: a new strategy for development of anti-infectives
16:40	Coffee break	
Therapeutics and technology 2 *Chair: Patrick O'Donoghue*		
17:10	IT17	**Wilfred van der Donk** Peptide-aminoacyl-tRNA ligases in natural product biosynthesis
17:30	ST23	**Jay Hesselberth** Nanopore sequencing of intact aminoacylated tRNAs
17:45	IT18	**Ana Crnkovic** Genetic code expansion as a tool for the development of novel protein-based nanosensors

18:05	IT19	**Jeffery Tharp** Synergistic genetic and chemical approaches to expand the molecular diversity of phage-displayed peptide libraries
18:25	ST24	**Allison Cale** Predictive modeling to define the locus heterogeneity of tRNA synthetase- related peripheral neuropathy
18:40	ST25	**Pengfei Fang** From mechanism to optimized inhibitors with enhanced selectivity targeting threonyl-tRNA synthetase
19:00	Dinner	
20:30	**Poster session with Cheese and Wine (Odd numbers)** *Sponsored by aTyr*	

Thursday, 2nd October

Chair: Karin Musier Forsyth

08:30	KL5	**IUBMB Keynote Lecture** **Hiroaki Suga** From genetic code reprogramming to therapeutic innovation

Transfer RNA 1
Chair: Oscar Vargas-Rodriguez

09:15		**Ioannis Tsagakis** FEBS Press
09:30	IT20	**Patrick O'Donoghue** Natural and therapeutic human transfer RNA anticodon variants
09:50	ST26	**Chris Ahern** Mechanisms and therapeutic applications of transfer RNA
10:05	IT21	**Lluís Ribas de Pouplana** Somatic tRNA mutations are abundant and rewire the genetic code in cancer and aging
10:25	Coffee break *Sponsored by Biosistemi*	

Transfer RNA 2
Chair: Xiao-Long Zhou

11:25	IT22	**Ya-Ming Hou** Ribosome selection of wobble-modified aminoacyl-tRNA exclusively by proofreading
11:45	IT23	**Haissi Cui** Exclusion of arginyl-tRNA synthetase from the multisynthetase complex causes defects in brain development
12:05	ST27	**Marisa Mendes** Temperature sensitivity of aminoacyl-tRNA synthetase variants and its implications for disease

12:20	IT24	**Ru-Juan Liu** Ultra-sensitive sequencing reveals extensive tRNA dynamics during cell differentiation
12:40	IT25	**Christopher Francklyn** Novel AIMP2 mutations cause neurodevelopmental defects through a loss-of-function mechanism linked to cell death in the developing zebrafish brain
13:00	**Career progression lunch**	
Disease *Chair: Leann Tilley*		
15:00	IT26	**Sunghoon Kim** Development of a unique domain embedded in tRNA synthetase for cancer therapy
15:20	IT27	**Susan Martinis** Mammalian leucyl-tRNA synthetase from liver cancer cells modulates cellular senescence and autophagy
15:40	ST28	**Bahaa Al-mhanawi** Elucidating the role of human multi-aminoacyl-tRNA synthetase complex (MSC) in hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL)
15:55	IT28	**Francesca Rapino** tRNA optimality as a determinant of cancer stemness in colorectal tumors
16:15	IT29	**Nam Hoon Kwon** Therapeutic potential of an anti-KARS1 antibody blocking monocyte migration in PAH models
16:35	IT30	**Pierre Close** tRNA modifications: a new vulnerability driving cancer heterogeneity & tumor immune response
16:55	Coffee break	

Closing ceremony		
17:30	IT31	**Closing lecture** **Paul Fox** EPRS1 goes nuclear
18:00		**Ita Gruic-Sovulj and Lluís Ribas de Pouplana** Prizes and closing
19:00	Dinner	

Friday, 3rd October

08:30	Departure	

Poster list

P1 **Engineered mistranslating tRNAs to suppress huntingtin production and aggregation in cellular models of Huntington's disease**

T.M.I. Bily, P. O'Donoghue

P2 ***In vitro* exploration of the multi-functional *Mycoplasma penetrans metS* gene product**

M. Bowman, R. Alexander

P3 **Translation perturbance in tRNA synthetase-defective trichothiodystrophy**

F. Brevi, A. Theil, A. Lehmann, S. Iben, D. Orioli, E. Botta

P4 **Defining molecular mechanisms and developing treatments for AARS2- related mitochondrial disease**

D. Brister, J. Collier, R. Horvath

P5 **From mitochondrial translation to cell cycle: unveiling a novel regulatory mechanism**

L.F. Costa Póvoas, N. Camacho, L. Ribas de Pouplana

P6 **Inactivation of Maf1, global negative regulator of tRNA transcription, leads to defects in translation elongation in yeast**

A. Armatowska, M. Cieśla, M. Świrski, J. Mikołajczyk, T. W. Turowski, E. Molestak, M. Tchórzewski, S. Bresson, M. Boguta

P7 **Structural basis for leucyl-tRNA synthetase assembly into the human multi-tRNA synthetase complex by isoleucyl-tRNA synthetase**

L.G. Dolce, B. Muller, A. Palencia

P8 **Dual role of single residue in the synthetic site of isoleucyl-tRNA synthetase: ensuring specificity of synthetic site reactions and maintaining communication with the editing site**

M. Dulic, N. Krpan, I. Gruic-Sovulj

P9 **The use of therapeutic suppressor tRNAs for Duchenne muscular dystrophy and dilated cardiomyopathy**

J. Oury, Y. Chen, A. Auld, S. McFarland, J. Khlghatyan, J. Wen, P. Eimon, P. Kaplan, E. Gardiner

P10 **Rapid growth and development of NARS1 patient directed research organization**

R. Heilmann

P11 **Exploring aminoacyl-tRNA synthetase-linked leukoencephalopathies: structural and biochemical insights into mitochondrial glutamyl-tRNA synthetase variants**

A.A. Dias, J.V. Ribeiro, B.J. Henriques

P12 **Assessing the impact of chimeric tRNAs on tumorigenesis and tumor development using *Drosophila melanogaster* cancer models**

J. Hernàndez i Feliu, L. Ribas de Pouplana

P13 **Targeting highly secreted tryptophanyl-tRNA synthetase: a potential theranostic for hypercytokinemic severe sepsis**

M. Jin, Y.T. Kim, K. Lee

P14 **HARS deficiency is rescued by tRNA supplementation in a humanized yeast model of Charcot-Marie-Tooth disease**

J. Kakadia, S. Wilhelm*, A. Beharry, R. Kenana, K. Hoffman, P. O'Donoghue, I. Heinemann*

P15 **Biogenesis of 5'-tRNA^{His} halves**

T. Kanaji, S. Kanaji, R. Shapiro, M. Horii, P. Schimmel, X. Yang

P16 **Exploring molecular adaptations of tRNA recognition in bacterial isoleucyl-tRNA synthetase type 2**

P. Kozulić, A. Brkic, M. Leibundgut, N. Ban, I. Gruic-Sovulj

P17 **Delivery of site-specific phosphorylated AKT1 to epithelial ovarian cancer cells prevents spheroid formation**

S. Kuang, P. O'Donoghue, I. Heinemann

P18 **Peptide–RNA coacervates enhance protoribosome function: implications for the origin of translation**

N. Kuprikova, V. G. Giacobelli, K. Hlouchova

P19 **An allelic series at R329 AARS1 reveals loss-of-function intolerance and variable dominant-negative effects**

S. Laureano Figueroa, A. Antonellis

P20 **Translation-mediated selective motor neuron degeneration in CMT-pathology associated with mutations in aaRS**

V. Lewandowski, D. Linnik, R. Thompson, B. Poolman, Z. Ignatova

P21 **NSUN7 is an RNA:m^{5}C catalytically inactivate protein that regulates spermatozoa flagellum assembly**

J. Li, W. Zhu, R. Liu

P22 **Deciphering the relationship between two arginyl-tRNA synthetases from the predatory bacteria *Herpetosiphon aurantiacus***

A. Madunic, I. Zivkovic, M. Mocibob, I. Gruic-Sovulj

P23 **Investigating the underlying molecular basis of CMT-causing AlaRS pathogenic alleles by using S. cerevisiae as a model organism**

M. Mahmood, N. X. Sun, I. Heinemann

P24 **Kinetic mechanism of *E. coli* IleRS determined by tRNA modifications**

H. McGuigan, Y. Hou

P25 **Selection of pyrrolysin-tRNA synthetase mutants for the cotranslational incorporation of boronic acid side chains into an Anticalin-type sugar-binding protein**

A.M. Mirwald, A. Skerra

P26 **Architectural basis of tRNA-dependent sterol aminoacylation**

H. Murayama, M. Nishimura, H. Gamper, Y. Hou, H. D. Becker, Y. Itoh, F. Fischer, O. Nureki

P27 **Analysis of Charcot-Marie-Tooth-associated tyrosyl-tRNA synthetase variants of unknown pathogenicity in *Saccharomyces cerevisiae***

N. Reynolds, M. Garcia

P28 **Inhibition of FARS with RNA from C9orf72 repeat expansion mutation associates with membrane protein alterations in ALS and FTD**

U. Čerček, J. Nimac, V. Župunski, B. Rogelj

P29 **Treatment of KARS1 deficiency with lysine supplementation in a pediatric patient: a three-year clinical update**

L. Rossetti

P30 ***P. falciparum* threonyl-tRNA synthetase in drug discovery: strategies for construct design and stabilization**

A. Rudnickiha, E. Parisini, A. Jirgensons

P31 **Bacterial seryl-tRNA synthetases as therapeutic targets: a fragment-based approach to inhibition**

L. Ruduša, S. Ozola, R. Aleksis, R. Bobrovs

P32 **Characterizing pathogenic mutations in asparaginyl-tRNA synthetase**

T. Samuels, I. Heinemann

P33 **Analyzing human aminoacyl-tRNA synthetase mutants using yeast**

B. Senger, S. Serre, H. Becker

P34 **Non-canonical functions of mitochondria-relocated cytosolic aminoacyl-tRNA synthetases in health and disease**

S. Serre Ibaceta, B. Senger, T.J. Nicholls, H.D. Becker, S. Friant

P35 **The use of genetic code expansion in the production of highly stable protein nanopores**

G. Spruk, G. Šolinc, G. Anderluh, A. Crnković

P36 **Human tRNA anticodon variants exceed read-through threshold for phenotypic rescue of an ALS-causative premature termination codon**

C. Ward, A. Beharry, K. Hoffmann, P. O'Donoghue

P37 **Development of an aminoacyl-tRNA synthetase for the incorporation of 7-hydroxycoumaryl-alanine**

K.B. Wolf, A. Skerra

P38 **tRNA-derived SINE repetitive elements regulate porcine embryonic genome activation**

Y. Wu, Y. Gao, Y. Miao, S. Gao

P39 **Inducing bacteria to self-destruct by hijacking the tRNA-charging process**

C. Tai, N. Ketprasit, C. Dogovski, W. Ye, M. Griffin, G. Carter, L. Tilley, S. Xie

P40 **Development of cyclic peptide inhibitors targeting system Xc via aminoacyl-tRNA synthetase–mediated synthesis and structural characterization by cryo-EM**

R. Zaitsu, Y. Ohno, F. Sano, Y. Kise, T. Kusakizako, H. Aikawa, T. Naohiro, S. Kitajima, H. Suga, O. Nureki

P41 **Expanding the genetic and clinical spectrum of neurological disorders associated with tRNA synthetase deficiencies in Russian patients**

E. Zakharova, S. Mikhailova, E. Saifullina, E. Larshina, Y. Itkis I. Nagornov, A. Ilyushkina, M. Sumina, F. Bostanova, I. Bychkov, O. Ivanova, T. Krylova

P42 **Deciphering tRNA repertoires and translation coordination during mouse early embryogenesis by ORACLE-tRNAseq**

Y. Li, X. Wang, R. Huang, H. Yu, Y. Wu, Y. Gao, X. Zhang, Y. Zhang

Keynote lectures

KL1

Ribosome upside down: co-translational protein biogenesis at the tunnel exit

N. Ban

Institute of Molecular Biology and Biophysics, ETH Zürich, Zürich, Switzerland

Our group aims to understand the cellular mechanisms underlying the production of functional proteins. This complex process requires an intricate interplay between the ribosome and a growing number of cellular factors that regulate translation and co-translational protein biogenesis. One of the key topics of our research, which combines structural, biochemical, and biophysical approaches, is the biogenesis of cytosolic and membrane proteins controlled by the nascent polypeptide–associated complex (NAC). I will present recent results that reveal how NAC coordinates the activity of a network of factors and enzymes at translating ribosomes to facilitate virtually all aspects of protein biogenesis, including nascent chain processing, modification, folding, and cellular localization.

Lentzsch et al., Nature (2024) 633:718-724
Gamerdinger et al., Science (2023) 380:1238-1243

KL2

Expanding the proteasome paradigm: uncovering the independent roles of the 20S core complex

M. Sharon

Weizmann Institute of Science, Rehovot, Israel

The discovery of the ubiquitin-26S proteasome pathway fundamentally transformed our understanding of intracellular protein degradation. While this system remains central to proteostasis, it is now evident that the 20S core proteasome also functions autonomously, independently of ubiquitin and ATP. Given that nearly half of all cellular proteasomes exist as free 20S complexes, this mode of degradation is far from rare—yet its mechanisms and biological significance are only beginning to emerge.

In this talk, I will present our recent findings that illuminate the distinct roles of the 20S proteasome. Through systematic substrate profiling, we identified endogenous targets enriched in RNA- and DNA-binding proteins with intrinsically disordered regions, many localized to the nucleus and stress granules. We also discovered a new family of protein modulators, which we term Catalytic Core Regulators (CCRs), that selectively tune 20S proteasomal activity. Extending these insights beyond the cell, we characterized the circulating 20S proteasome in blood. This uncapped complex displays unique post-translational modifications, enhanced caspase-like activity, and enrichment in immunoproteasome subunits—hallmarks of adaptation to extracellular conditions. Together, our studies redefine the 20S proteasome as a versatile and autonomous degradation system, essential for safeguarding proteostasis across both intracellular and extracellular environments, with broad implications for stress adaptation, immune function, and disease.

KL3

RNA condensates at the nexus of cellular stress, disease and aging

S. Alberti

Technische Universität Dresden, Biotechnology Center (BIOTEC), Dresden, Germany

Biomolecular condensates play a crucial role in organizing cellular architecture and regulating essential functions. Among these, ribonucleoprotein (RNP) granules represent a distinct class of condensates that assemble from RNA-binding proteins and RNA. In this talk, I will explore how the concept of biomolecular condensates has transformed our understanding of RNP granules and their links to disease, aging, and cellular stress responses.

I will introduce in vitro reconstitution systems based on phase separation, which now enable us to reconstruct RNP granules in a test tube. Leveraging these systems, along with advanced imaging techniques and biophysical approaches, we have uncovered key molecular principles governing RNP granule assembly. These include the driving forces and amino acid determinants of condensation, the conformational changes underlying assembly, and the molecular mechanisms that regulate condensate formation and function.

Furthermore, I will discuss how the condensate framework has provided new insights into the functional roles of RNP granules. Specifically, I will highlight how cells exploit condensate formation to sense environmental changes and regulate fundamental processes such as protein synthesis. By dissecting these mechanisms, we gain a deeper understanding of how biomolecular condensates contribute to cellular homeostasis and adaptation.

KL4

Mitochondrial tRNA synthetase disorders: moving towards therapy

R. Horvath

Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom

Mitochondrial aminoacyl-tRNA synthetase (mt-aaRS) disorders are a group of rare, inherited diseases that impair mitochondrial protein synthesis by disrupting the attachment of specific amino acids to their corresponding mitochondrial tRNAs. Although mt-aaRS enzymes are ubiquitously expressed in all cell types, mutations in these genes lead to highly variable, tissue-specific clinical manifestations. Commonly affected systems include the central and peripheral nervous systems, skeletal muscle, heart, and other organs. We have generated a phenotypic database of mt-aaRS diseases to support evaluation of variants of unknown significance (VUS) in patients with novel variants in these genes. Using patient-derived cellular models and zebrafish, we investigate the molecular mechanisms underlying tissue-specific pathologies, enabling a deeper understanding of disease biology and the identification of potential therapeutic targets. We have initiated a clinical trial with targeted amino acid supplementation in children with mutations in EARS2, AARS2, and DARS2. This therapeutic strategy is designed to improve deficient aminoacylation activity and improve mitochondrial function. Our research highlights the complexity of mt-aaRS disorders and the importance of precision approaches in modelling and treating these conditions.

KL5

From genetic code reprogramming to therapeutic innovation

H. Suga

The University of Tokyo, Tokyo, Japan

In the last nearly three decades, my labotorary devised technologies for drastic reprogramming of the genetic code in vitro. The technologies involve flexizymes, FIT (flexible *in-vitro* translation) system, and RaPID (random nonstandard peptides integrated discovery) system. These technologies have enabled us to investigate the uncovered potentials of ribosome and tRNAs, the application to pseudo-natural macrocyclic peptides, and their therapeutic innovation. This lecture discusses the history and most recent outcomes of our research programs.

Invited talks

IT1

Boolean analysis of human tRNA synthetase splice variants

P. Schimmel

Scripps Research, San Diego, CA, United States of America

We show how use of a Boolean formulation enables us to efficiently record and combine the over 200 known aaRS splice variants. This formulation enables us to construct tissue- and disease-specific ensembles of SVs, using public databases. The results give new insights into a complex system of how SVs work to maintain cellular homeostasis.

IT2

Surprising enzymatic activity of OB-fold containing multi-aminoacyl-tRNA synthetase adaptor proteins

K. Musier-Forsyth

The Ohio State University, Columbus, OH, United States of America

In eukaryotes, aminoacyl-tRNA synthetases (ARSs) form multi-ARS complexes (MSC) that are assembled with several non-synthetase host factors believed to serve primarily adaptor or scaffolding roles. The Trypanosoma brucei MSC includes two non-ARS host proteins with oligosaccharide/oligonucleotide-binding (OB) folds — MSC-associated protein 1 (MCP1) and MCP2—and a third factor, MCP3, which we previously demonstrated was an Ala-tRNA deacylase with homology to the INS superfamily of editing domains. Surprisingly, MCP1 also deacylates Ala-tRNAs despite lacking known tRNA-editing domain homology. Deletion studies revealed that the OB-fold houses the catalytic pocket and mutation of any one of three conserved residues (K326, R331, S335) abolished activity. Assays with *Saccharomyces cerevisiae* Arc1p, an MCP1 homolog and a component of the smallest known three-member MSC, revealed that this adaptor protein also displays robust Ala-tRNA deacylation activity. Human AIMP1 is another OB-fold homolog that is housed in the human MSC and also robustly deacylates Ala-tRNAs. Thus, deacylation activity by OB-fold containing MSC adaptor proteins is conserved across organisms and these factors constitute an entirely new family of tRNA editing domains.

IT3

Structural basis for tRNA-dependent lipid aminoacylation

M. Nishimura[1], H. Murayama[1], Y. Itoh[1], Y. Kise[1], H. B. Gamper[2], Y-M. Hou[2], H. D. Becker[3], H. Roy[4], F. Fischer[3] , O. Nureki[1]

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Aminoacylated transfer RNAs (aa-tRNAs) act as a donor of activated aminoacyl moiety, not only in translating ribosome, but also to diverse acceptor molecules by enzymes called aa-tRNA transferases (ATTs). Both in prokaryotes and eukaryotes, ATTs are often bi-functional enzymes containing aminoacyl-tRNA synthetase domain and Gcn5-N-acetyltransferase (dGNAT)-like fold ATT domain for tRNA-mediated rapid substrate handover to increase the catalytic efficiency. Here we present two ATTs that transfer aminoacyl moiety to lipids.

MprF is a prokaryotic bifunctional enzyme that synthesizes an aminoacyl-phosphatidylglycerol (aa-PG) by transferring a Lys or Ala from an aminoacyl-tRNA to PG and flips the aa-PG to the extracellular leaflet of the membrane, ensurring bacterial resistance against cationic antimicrobial peptides produced by host. We solved the cryo-EM structures of full-length *Rhizobium etli* MprF (ReMprF) in tRNA-free and Lys-N-tRNA^{Lys}-bound forms. The Lys-N-tRNA^{Lys}-bound structure showed that a single aa-tRNA molecule binds to the ReMprF homodimer, with its anticodon stem loop inserted between the transmembrane flippase domain and the aa-PG synthase (aa-PGS) domain of one protomer. Lys-N-tRNA^{Lys} binding detaches the aa-PGS domain from the transmembrane flippase domain, allowing the aa-PGS domain to access and accommodate the substrate PG from the bulk lipid membrane and synthesize aa-PG.

In higher fungi, including human and plant pathogens, we newly identified an ergosteryl-3β-*O*-(L)-amino acid (Erg-aa) synthase (ErdS), which conjugate Asp to sterols and is essential for proper hyphal growth, sporulation and spore germination. The Cryo-EM structure of ErdS complexed with non-hydrolyzable Asp-N-tRNA^{Asp} revealed a tRNA-guided intramolecular aminoacyl transfer mechanism between two functional domains: the CCA end of tRNA^{Asp} undergoes a large displacement to

reach the aa-tRNA transfer active site, while tRNA elbow is clamped by a long extension of N-terminal α-helix. The present structural and mutational analyses demonstrate a unique regulatory mechanism of tRNA-dependent sterol modification and provide novel insights into fungal membrane dynamics, highlighting potential novel targets for antifungal therapies.

IT4

Is speed the key? Exploring divergence in bacterial tRNA^{Ile} recognition

I. Gruic-Sovulj

University of Zagreb, Faculty of Science, Zagreb, Croatia

Isoleucyl-tRNA synthetase (IleRS) charges tRNA^{Ile} with isoleucine. In bacteria, two types of IleRS exist, each with distinct tRNA recognition domains. IleRS1 features an essential C-terminal Zn-binding domain, which groups with treble clef fingers. In contrast, IleRS2 lacks the Zn-finger motif but instead has a distinct C-terminal tRNA-binding domain, which likely arose by domain duplication. Using multiple approaches, we explored the divergence in tRNA^{Ile} recognition between the two bacterial IleRS types. We found that their evolutionarily distinct C-terminal domains serve the same role in tRNA recognition by reading the first base of the anticodon. However, IleRS1 and IleRS2 read the anticodon by different mechanisms, leading to less stringent anticodon recognition by IleRS1. We further discovered that only IleRS2, within its tRNA-binding domain, has an insertion that makes backbone-specific interactions with the tRNA^{Ile} elbow. Disruption of these interactions unexpectedly increases the turnover of IleRS2. IleRS2 is more commonly found in slower-growing bacteria, whereas IleRS1 predominantly occurs in faster-growing ones (Zanki et al., Protein Science (2022) 31:e4418). We speculate that the requirement for faster turnover drove the assembly of the distinct tRNA-binding architecture in IleRS1.

IT5

Chiral proofreaders, their variants and their functional roles

R. Sankaranarayanan

CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India

Despite the presence and biological role of D-amino acids, the fundamental issue of how proteins are made only with the L-chiral entities was largely ignored. Over the last two decades, it has become clearer as to how multiple 'Chiral Checkpoints' work in concert to avoid D-amino acids from getting incorporated into proteins. Our work over the past decade has shed light on how chiral proofreading systems have played critical roles in important evolutionary transitions. These studies have highlighted the roles of different variants such as DTD2 (D-aminoacyl tRNA deacylase 2) and Animalia-specific tRNA deacylase (ATD) in in key evolutionary transitions such as land plant adaptation and multicellularity. DTD2 is an archaeal origin enzyme that is horizontally transferred to land plant ancestors wherein it clears acetaldehyde modified adducts on D-aminoacyl tRNA. This property of the enzyme is being considered as a potential candidate for developing stress resistant plant varieties. The other variant ATD was originally identified as a proofreading factor for tRNA misaminoacylation. However, the error inducing tRNA species seems to have appeared a few hundred million years later than ATD suggesting a possibility of another role for its evolution. My talk will cover some of the above aspects that are the focus of our current efforts in the laboratory.

Kuncha et al., J. Biol. Chem. (2019)
Kumar et al., FEBS Letters (2022)
Mazeed et al., Science Advances (2021)
Kuncha et al. eLife (2020)
Kumar et al., eLife (2024)

IT6

Pre-LUCA protein evolution

K. Hlouchova, V. Giacobelli

Charles University, Prague, Czech Republic

All extant cells known to humankind build proteins from the same 20 coded amino acids. Is this canonical alphabet a prerequisite for life to be as successful as it has been on our planet or could protein structures and functions depend on a different/smaller set of amino acids?

We report structural and functional propensities of proteins and peptide libraries built from different subsets of both canonical and non-canonical amino acids, that could represent different hypothetical stages of the prebiotic-to-biological sequences. The study of origins of life implies that earlier cells functioned with a smaller alphabet, which was selected from a pool of prebiotically plausible (canonical and non-canonical) amino acids before the fixation of the Central Dogma. Our work implies that the “early” canonical amino acids that were selected from the prebiotic environment have a higher structure-forming propensity than possible alternatives. Despite lacking positively charged and aromatic residues, proteins composed from such “early” components would be prone to structure formation but also capable of interacting with organic and inorganic cofactors. On select examples, we observe that such binding can significantly assist with early protein folding as well as with catalytic and binding propensities.

Our work indicates that protein folding propensity was an important factor during the earliest stages of the genetic code evolution. A reduced acidic alphabet would be sufficient to build small proteins, sometimes capitalizing on interactions that are less frequent or rare in today’s biology.

IT7

Homocysteine thiolactone from translation proofreading is an endogenous ligand for cell-cell signaling by the receptor SdiA

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Several widespread mechanisms enable cellular communication and coordinate the behavior of bacterial populations. The modules responsible for cellular communication are often found in matching pairs: an enzyme synthesizing a signal molecule and a specific receptor capable of decoding it. A notable exception is *Escherichia coli* SdiA, a homolog of known N-acyl homoserine lactone (AHL) receptors for which no corresponding AHL synthase exists. SdiA is an orphan receptor that enables cross-talk by sensing AHLs from other bacterial species. In this work, we investigated if homocysteine thiolactone (HTL), an AHL-like molecule arising from a proofreading reaction of methionyl-tRNA synthetase (MetRS) to correct misactivation of homocysteine, participates in E. colicommunication through SdiA. A comprehensive analysis of MetRS variants producing different levels of HTL showed more extended lag periods due to lower HTL levels, which were restored by HTL supplementation. SdiA mutants abolished the regulation of the transition from the lag phase to the exponential phase induced by HTL. Growth phenotypes were HTL- specific and were not induced by supplementation of other non-endogenous AHLs previously characterized as SdiA ligands. The gene expression of gadY and rmfregulated by SdiA was modulated by HTL supplementation. Through differential scanning fluorimetry, we show the SdiA-HTL interaction in vitro. Together, our observations describe HTL as an SdiA ligand capable of modulating cell-cell communication in bacteria.

IT8

Translational fidelity and the integrated stress response in eukaryotes

H. Zhang, P. Murphy, J. Ling

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The integrated stress response (ISR) is an adaptive mechanism in response to multiple stresses in eukaryotes. However, chronic activation of the ISR contributes to the development of multiple diseases, such as neuropathies. We have recently shown that Ser misincorporation into Ala and Thr codons, resulting from either aaRS-editing defects or mutations in tRNAs, activates the ISR. We also demonstrate that activation of the ISR by Ser mistranslation does not depend on the accumulation of uncharged tRNAs but rather requires the P stalk associated with the ribosome, implying that ribosome stalling and collision are involved. To further investigate the role of translational fidelity in eukaryotic stress responses, we created yeast strains with high and low ribosomal fidelity. Unexpectedly, hyper-accurate ribosomes activate the ISR. We provide further mechanistic insights into how translational fidelity impacts fitness and stress responses.

IT9

Vacuolar echoforms of yeast cytosolic aminoacyl-tRNA synthetases are modulating components of TORC1

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The yeast Tor Complex 1 (TORC1) is a key signaling component regulating a wide range of processes spanning from ribosome biogenesis to autophagy through phosphorylation of multiple target proteins by its central kinase called Tor1 (EMBO J. (2017) 36 (4):397-408). It is located at the surface of the vacuole where it evaluates nutrient availability among which the level of intracellular amino acids (aa).

The mechanisms by which TORC1 senses and responds to the availability of each of the 20 proteinogenic aa is poorly documented except for leucine (Leu). Leu is detected by the vacuolar echoform of leucyl-tRNA synthetase (LRS), that relays Leu content to TORC1 through dynamic binding to the EGO complex (Mol. Cell (2012) 46 (1):105-10).

Using a strain that we engineered to express a vacuolar-restricted Split-CFP (Vac-Split-CFP), we found that 18 of the 20 cytosolic aminoacyl-tRNA synthetases (aaRS) and Arc1 could relocate at the surface of vacuole (vacuolar echoforms) and thus possibly interact with TORC1. By immunoprecipitation experiments we confirmed that most of these aaRSs indeed dynamically bind to Kog1, a core component of TORC1, in an aa-dependent manner.

We then focused on the interplay between TORC1 and the 3 components of the AME multi-synthetase complex using a combination of different approaches that

allowed us to confirm an increased binding of ERS, MRS and Arc1 to TORC1 components under nutritional stress. We also showed that AME components bind to TORC1 with high affinity and have data pointing at which domains and components of TORC1 might mediate this interaction. Finally, by monitoring the phosphorylation of downstream targets of TORC1 we could measure the activity of the Tor pathway in presence of a collection of different variants of MRS, ERS, and Arc1. Altogether, our data show that the three components of the AME complex are key regulators of TORC1 activity in response to nutritional changes.

IT10

WHEP domain of glycyl-tRNA synthetase regulates neuropilin 1 binding and vascular permeability

X. Yang

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Aminoacyl-tRNA synthetases (aaRSs) charge tRNAs with their cognate amino acids, ensuring accurate translation of the genetic code from mRNA to protein. During eukaryotic evolution, aaRSs acquired additional domains with unclear functions, including the WHEP domain, a two-helix bundle found in several eukaryotic aaRSs such as glycyl-tRNA synthetase (GlyRS, encoded by GARS1). We generated Gars1ΔWHEP mutant mice lacking exon 2, which disrupts most of the WHEP domain. Homozygous Gars1ΔWHEP/ΔWHEP mice exhibited late embryonic or neonatal lethality, with delayed lung development, characterized by reduced airway dilation (inflatability), and increased vascular leakage. Disruption of the WHEP domain did not impair tRNA aminoacylation but inhibited the free release of GlyRS from cells. Instead, GlyRSΔWHEP was found in the membrane fractions and showed a stronger interaction with the extracellular region of neuropilin 1 (Nrp1) receptor compared to full-length GlyRS. This aberrant interaction enhanced Nrp1's endocytic activity and significantly reduced the localization of the Nrp1 interactor VE-cadherin at the adherens junctions of endothelial cells. A heterozygous knockout of Nrp1 in the Gars1 ΔWHEP/ΔWHEP mice partially rescued body weight and vascular permeability defects. This study establishes a physiological role for the GlyRS WHEP domain in lung development and its regulation of GlyRS-Nrp1 interaction and vascular permeability.

IT11

Role of aminoacyl-tRNA synthetases in oxidative stress response

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Interplay of translation and oxidative stress was studied on two examples: plant seryl-tRNA synthetase (SerRS) and bacterial isoleucyl-tRNA synthetase (IleRS). A unique disulfide bond was identified in the crystal structure of *Arabidopsis* cytosolic SerRS involving cysteines evolutionarily conserved in all green plants. Both cysteines are important for protein stability, but with opposite effects, while their microenvironment promotes disulfide bond formation in oxidizing conditions. The activity of the wild-type showed resistance to oxidation with H_2O_2, while activities of cysteine-to-serine mutants were impaired, indicating that the disulfide link may enable the protein to function under oxidative stress conditions which can be beneficial for an efficient plant stress response. Mistranslation usually has negative consequences, however, moderate levels of mistranslation may have a beneficial effect. IleRS can misrecognize valine (Val) and norvaline (Nva), amino acids structurally similar to isoleucine, but possesses an editing domain (ED) domain to correct such errors. Incubation of an *Escherichia coli* strain that expresses IleRS with a non- functional ED domain in a medium with Val or Nva caused increased levels of mistranslation but also enabled bacteria to better cope with subsequent oxidative stress conditions. Using quantitative mass spectrometry, differential protein expression in samples with or without Val or Nva was analyzed. Val mistranslation resulted in upregulation of arginine biosynthetic process, while Nva mistranslation had a major impact on translation process. Both Val and Nva mistranslation caused upregulation of peptide transport across plasma membrane and downregulation of glutamate metabolism and enterobactin biosynthesis, involved in iron transport. Several proteins involved in protein folding and stress response were also increased. Identified cellular processes may enable improved adaptation of mistranslating bacteria to oxidative stress.

IT12

How the analysis of pathology-related variants shed light on fundamental process: the case of PARS2 variants

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Though mitochondrial aminoacyl-tRNA synthetases (ARS2) are widely expressed and critical for mitochondrial genome translation, ARS2 gene variants are associated with various neurological disorders. PARS2 deficiency, caused by mutations in the nuclear gene encoding mitochondrial prolyl-tRNA synthetase (ProRS), was reported in few patients. Here, we describe two unrelated patients with epileptic encephalopathy carrying biallelic PARS2 variants: one previously known and one novel predicted as "likely benign" or "variant of uncertain significance" by conventional in silico predictors. By conducting a phenotypic study, we established that the patients' clinical presentation aligns with that of a cohort of 22 patients previously reported with PARS2 deficiency. By using comparative protein structure modeling and detailed clade-specific sequence conservation analysis, we found that the novel variant actually lies within a previously unrecognized zinc-binding domain (ZBD), structurally similar to the well-characterized and essential ZBD of cytosolic ProRS. Our findings highlight the limitations of current variant prediction tools for ARS2 genes and emphasize the importance of integrative structural and evolutionary analyses. Our study broadens the genetic spectrum of PARS2-related disorders, provides a comprehensive description of the associated phenotypes and offers new insights into the structural and functional properties of PARS2.

IT13

The nuclear function of TyrRS as a mediator of stress response in the Charcot-Marie-Tooth neuropathy

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Mutations in the YARS1 gene encoding the cytoplasmic tyrosyl-tRNA synthetase (TyrRS) cause a subtype of Charcot-Marie-Tooth disease (CMT), the most common inherited peripheral neuropathy that remains incurable. TyrRS is an evolutionarily conserved and ubiquitously expressed enzyme that catalyzes the attachment of tyrosine to its cognate tRNA. Yet, the mechanisms by which TyrRS dysfunction leads to neurodegeneration remain poorly understood. In addition to its canonical translational function exerted in the cytoplasm, TyrRS translocates to the nucleus in response to oxidative stress, where it activates transcriptional reprogramming mediating cell survival. Notably, the CMT-causing variants disrupt this protective nuclear function despite preserving the aminoacylation activity of the mutant protein. Recent studies have implicated the activation of the integrated stress response (ISR) -cellular signaling pathway triggered by amino acid deprivation, oxidative and endoplasmic reticulum stress or viral infection- as a potential unifying pathomechanism across several CMT-causing tRNA synthetases, including TyrRS. In this study, we set out to investigate the putative role of nucleus-localized TyrRS in ISR activation. Using Drosophila and cell-based TyrRS-CMT models, we demonstrate that the ISR is involved in the toxicity caused by the CMT mutations, and the nuclear function of TyrRS is required for exerting this defense response.

IT14

tRNA-based therapies: roadblocks en route to their establishment as treatment for diverse pathologies

Z. Ignatova

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For many genetic disorders, including CMT disease linked to mutations in several aminoacyl-tRNA synthetases and pathologies linked to nonsense mutations, tRNA-based therapies emerge as a promising therapy. I will discuss both type of administrations, as lipid nanoparticle (LNP) or as adeno-associated viral (AAV) formulations for single or repeated treatment modalities, along with the considerations in tRNA design to establish them as a potent and safe therapy.

IT15

Advancing a therapeutic platform based on tRNA synthetases for treatment of fibrotic lung diseases

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Extracellular functions of tRNA synthetases represent a novel and expanding area of biology, offering new opportunities for physiological modulation and therapeutic intervention. We have established a platform to systematically advance domains of tRNA synthetases from conceptual discovery to clinical-stage drug candidates. This approach integrates foundational insights from our early research with current scientific understanding of tRNA synthetase evolution, protein structure, alternative splicing, and tissue-specific expression. Through this process, we identify and develop biologically active, extracellular domains with therapeutic potential. Our most advanced candidate, efzofitimod, is an immunomodulatory protein derived from a naturally occurring splice variant of histidyl-tRNA synthetase selectively upregulated in human lung tissue. This molecule is currently being evaluated in two clinical trials of fibrotic interstitial lung disease. Building on this foundation, we are expanding our pipeline with additional tRNA synthetase-derived therapeutic candidates. One of these, ATYR0101, is based on a domain that is released from aspartyl-tRNA synthetase by proteolysis upon macrophage activation that binds latent TGFβ-binding protein 1, a key regulator of TGFβ activation. By selectively inducing apoptosis in pathogenic myofibroblasts in a TGFβ-dependent manner, ATYR0101 addresses a core cellular mechanism of fibrosis. In addition to demonstrating efficacy in preclinical models of fibrosis in both lung and kidney, spatial transcriptomic analyses demonstrate that ATYR0101 exhibits a transcriptional signature distinct from current standard- of-care therapy, supporting its potential as a differentiated approach for treating advanced fibrotic disease. Our tRNA synthetase-based therapeutic platform creates a deeper understanding of the diverse biological functions of these proteins extracellularly and supports their potential development across a wide range of disease areas.

IT16

Structural studies on protein–RNA complexes and applications for the development of novel therapeutics

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Our team employs an integrated structural biology and biophysical approach, combined with AI-based predictions and functional studies, to investigate the inhibition mechanisms of novel protein and RNA targets involved in human diseases, to develop new therapeutics. We are interested in aminoacyl-tRNA synthetases (AARS), which are responsible for attaching the correct amino acid to the cognate tRNA (a process called aminoacylation while preventing the attachment of incorrect amino acids to tRNAs (known as editing). AARSs are valid therapeutic targets because they are essential for protein translation and show differences between microbial pathogens and humans, which can be exploited for drug discovery. Using a structure-based approach, we have identified inhibitors of Leucyl-tRNA synthetase (LeuRS) and optimise their properties to allow the progression into clinical studies. Specifically, benzoxaborole inhibitors of bacterial LeuRS present excellent clinical properties against Gram-negative bacterial pathogens, *M. tuberculosis* and pathogenic nematodes that depend on symbiotic bacteria such as *Wolbachia*.

Another key area of our research targets the molecular machinery involved in 3'-end processing and polyadenylation of mRNA. In eukaryotes, pre-mRNA maturation involves large protein-RNA complexes that cleave the 3'-end of nascent mRNA transcripts and add a poly(A) tail, a step critical for mRNA stability, export to the cytoplasm, and translation. The cleavage is catalyzed by the endonuclease CPSF3, while polyadenylation is carried out by Poly(A) Polymerase α (PAP α). We are exploiting the essentiality of these enzymes to develop inhibitors with potential therapeutic applications for treating infectious diseases caused by eukaryotic pathogens, such as *Plasmodium* spp. and *Cryptosporidium* spp. Examples of novel inhibitors targeting CPSF3 and PAPα in eukaryotic parasites will be presented.

IT17

Peptide-aminoacyl-tRNA ligases in natural product biosynthesis

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Ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a large class of peptide natural products that are made in all domains of life. Their biosynthetic genes are ubiquitous in the currently sequenced genomes, their structural and functional diversity is vast, and they are increasingly recognized for their involvement in protecting against or causing human disease. This presentation will discuss examples of genome mining that discovered an unexpected utilization of various aminoacyl-tRNAs for the non-templated extension of peptides. Peptide aminoacyl-tRNA ligases (PEARLs) add amino acids to the C-terminus of ribosomally synthesized peptides. Their mechanism will be discussed as well as their likely evolutionary origin and engineering applications.

IT18

Genetic code expansion as a tool for the development of novel protein-based nanosensors

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The expansion of the genetic code and the co-translational insertion of non-canonical amino acids (ncAAs) holds great potential for biotechnological applications. One of these applications is the creation of modified biological nanopores based on natural proteins that form nanoscale apertures in lipid bilayers. Biological nanopores, best known for their use in DNA sequencing, have been adapted for single-molecule biosensing for a variety of analytes. However, certain analytes are still difficult to analyze due to their size, sample heterogeneity, or the inability of biological nanopores to withstand the harsh conditions sometimes required to analyze the sample. Here, we show that site-specific insertion of ncAAs can facilitate the creation of protein-based nanosensors tailored to the detection of specific molecular targets. Through an in- depth biophysical and structural analysis of modified biological nanopores, we demonstrate the assembly and biosensing capability of ncAA-containing nanopores. The nanopores in question can be used for the detection of certain ions that occur as environmental pollutants, as well as nanopores that can withstand large amounts of chemical denaturants, which is beneficial in the analysis of full-length folded protein analytes. Overall, our approach demonstrates the versatility with which genetically incorporated ncAAs can enrich and improve detection strategies in nanopore biosensing.

IT19

Synergistic genetic and chemical approaches to expand the molecular diversity of phage-displayed peptide libraries

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Peptides are an attractive scaffold for therapeutic development due to their unique ability to combine desirable features of both small molecules and protein-based biologics. Like small molecules, peptides are synthetically accessible and can effectively penetrate tissues, yet they offer the high binding affinity and specificity characteristic of biologics. A key advantage of peptides lies in the availability of powerful screening technologies, such as phage display, which enables the rapid identification of novel peptide ligands from vast, combinatorial peptide libraries. However, conventional phage display libraries are limited to the 20 natural amino acids, which lack useful chemical functionalities found in non-ribosomal peptides and synthetic pharmaceutics. As a result, the full potential of phage display in therapeutic peptide drug discovery remains underutilized. To address this limitation, we have developed a strategy that combines unnatural amino acid (uAAs) mutagenesis with chemical posttranslational modification, to rapidly expand the molecular diversity of phage-displayed peptide libraries. This platform employs engineered, orthogonal aminoacyl-tRNA synthetases to site-specifically incorporate reactive uAAs into peptide libraries for subsequent modification using phage-compatible, bioorthogonal chemistry. In this presentation, I will discuss the development of this enhanced phage display platform and highlight early efforts to apply it toward the discovery of peptide-based inhibitors of therapeutically relevant enzymes.

IT20

Natural and therapeutic human transfer RNA anticodon variants

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Diverse cells, including human cells, tolerate surprisingly high levels of translation error, and human genomes encode missense suppressor tRNAs that cause mistranslation. We characterized natural human variants as well as synthetic missense and nonsense suppressor tRNAs that cause errors in protein synthesis or reassign sense or stop codons in archaea, bacteria, yeast, and mammalian cells. Suppressor tRNAs inspired by those we discovered can also suppress mutations in disease-causing alleles. We repurposed natural and generated synthetic tRNAs to suppress causative mutations in models of Huntington's disease (HD), amyotrophic lateral sclerosis, and frontotemporal dementia (FTD). We showed that missense suppression of glutamine codons can reduce HD-causing poly-glutamine levels and aggregates in human and murine neuroblastoma cells. In an FTD model, we observed 60% readthrough of a progranulin allele containing a premature stop codon using a human $tRNA^{Arg}$ nonsense suppressor. We found that different human $tRNA^{Arg}UCG$ isodecoders, bearing the same engineered anticodons (G36A) but with minor differences in the tRNA sequence mediate significant and differential nonsense suppression that depends on the tRNA gene sequence and the cell in which it is expressed. The suppressor tRNAs significantly outperformed an aminoglycoside nonsense suppressor (G418) in efficacy, tolerability to the cells, and in translation fidelity as we validated by mass spectrometry. Our studies show that both missense and nonsense suppressor tRNAs can correct or ameliorate genetic defects that cause disease in humans.

IT21

Somatic tRNA mutations are abundant and rewire the genetic code in cancer and aging

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The human genome contains hundreds of genes coding for tRNAs (tDNAs). It has been reported that tDNAs in the human germinal cell line experience intense mutagenesis associated with RNA polymerase III's transcriptional activity. This accumulation of mutations in germinal cells affects only the untranscribed upstream and downstream regions of tDNAs, indicating that these mutations are under strong purifying selection. The mutagenic status of tDNAs in somatic cells has been reported but not characterized in detail.

Here we first map all tDNAs in the T2T version of the human genome, and use this information to study the incidence of somatic mutagenesis at these loci. As previously reported, tDNAs can be found as isolated genes or as part of large gene clusters. Combining our data with available POLR3D ChIP-seq and biotin-capture of nascent RNA data, we find that tDNAs in clusters exhibit higher activity levels than isolated genes.

We then characterized in detail the somatic mutations affecting tDNAs in human tissues and found that somatic mutational degeneration of human tDNAs is a prevalent phenomenon. Internal regions of tDNAs constitute hotspots of somatic mutagenesis whose intensity is directly linked to the transcriptional activity of each gene. The mutational load at tDNAs is not found at other genes transcribed by Pol III, and accumulates in tDNAs at rates up to nine-fold higher than in protein-coding genes. Somatic mutations at tDNAs happen in both healthy and cancer cells, but the mutational signatures identified in human tumors are not identical to those involved in healthy tissues. Mutations at tDNAs accumulate with age and generate mutant chimeric tRNAs capable of introducing specific and ubiquitous amino acid substitutions throughout the proteome.

IT22

Ribosome selection of wobble-modified aminoacyl-tRNA exclusively by proofreading

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High-fidelity selection of aminoacyl-tRNAs (aa-tRNAs) that match the codon in the ribosome decoding site is essential for life. This high-fidelity selection against mismatches of natural nucleotides at any position of the anticodon-codon pairing is achieved in two stages – the initial selection and the subsequent proofreading. How the ribosome responds to aa-tRNAs that contain wobble modifications of anticodon-codon pairings is unknown. An example is the Gram-negative cmo^{5}U34-modified anticodons that read both the cognate A-ending codons and the near-cognate C-ending codons. Here we compare ribosome selection of *E. coli* tRNA Pro(cmo^{5}UGG) and its structural homolog Pro(ho^{5}UGG), a key intermediate in the biosynthesis of cmo^{5}U34. A mutant strain that terminates at ho^{5}U34 is sensitive to stress and is deficient in reading codons of CCC vs. CCA. Kinetic assays with reconstituted ribosomes show that discrimination against the CCC codon is executed exclusively at the proofreading stage. Single-molecule assays support that Pro(ho^{5}UGG) during proofreading is dynamically more labile relative to Pro(cmo^{5}UGG). Thus, unlike discrimination against mismatches of natural nucleotides, discrimination against mismatches of wobble-modified nucleotides is solely by ribosome proofreading, emphasizing the importance of proofreading as the final fidelity checkpoint before the aa-tRNA leaves the decoding site.

IT23

Exclusion of arginyl-tRNA synthetase from the multisynthetase complex causes defects in brain development

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Due to their central role in protein synthesis, mutations in aminoacyl-tRNA synthetases can cause severe disease phenotypes. Biallelic or compound heterozygous mutations in aminoacyl-tRNA synthetase genes have been found causative for developmental brain disorders, specifically hypomyelinating leukodystrophies.

While many of these mutations disrupt the enzymatic activity, a subset of mutations is found in domains which mediate the integration of aminoacyl-tRNA synthetases into the multisynthetase complex. Indeed, the most common mutations in Arginyl-tRNA synthetase (ArgRS) led to an altered ratio of free vs multisynthetase-bound ArgRS, without affecting tRNA charging. We were interested in investigating why neural cells are especially vulnerable, and which cell types are predominantly affected. To this end, we developed a mouse model, which has a forebrain specific deletion of full-length, multisynthetase-complex bound ArgRS, and instead expresses only a free-standing form of ArgRS. In these animals, we found a severe reduction of brain volume, reduced weight, and coordination issues. Preliminary studies suggest that oligodendrocyte differentiation is reduced, however, myelination seems proportional in the adult brain. We are currently developing new strategies to probe whether protein synthesis is affected, and which cell types are predominantly experiencing alterations in their proteome.

Overall, we strive to investigate the role of multisynthetase-complex bound ArgRS in neurodevelopment and understand the contribution of aminoacyl-tRNA synthetase organization to neural protein synthesis.

IT24

Ultra-sensitive sequencing reveals extensive tRNA dynamics during cell differentiation

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Mammalian genomes encode hundreds of tRNA genes, but how individual tRNAs regulate developmental precision and cellular identity specification remains largely unclear. The complex three-dimensional structure of tRNAs and numerous post-transcriptional modifications on tRNAs have long rendered tRNA sequencing a technical challenge. Here, we introduce an ultra-low input sequencing approach that simultaneously detects full-length tRNAs, certain modifications, and tRNA-derived fragments (tdRs) using as few as five cells. Applying this strategy, we conducted a comprehensive analysis of tRNA profiles in 28 different cell types within the same mice tissue, and revealed dynamic remodeling of tRNA and tdR expression during lineage commitment and differentiation. We identify over 50 tRNA isodecoders with strong cell type-specific expression patterns, suggesting specialized regulatory roles during differentiation. Additionally, we observed significant changes in the expression ratios of specific tdRs, as well as variations in certain modifications during differentiation. These findings provide new insights into the diversity and functional specificity of the tRNA pool in mammalian development.

IT25

Novel AIMP2 mutations cause neurodevelopmental defects through a loss-of-function mechanism linked to cell death in the developing zebrafish brain

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Aminoacyl-tRNA synthetase multifunctional interacting protein 2 (AIMP2) is a component of the eukaryote specific multisynthetase complex (MSC), a large macromolecular assembly comprised of eight aminoacyl-tRNA synthetases (ARS) and three non-enzymatic scaffolding components known as aminoacyl-tRNA synthetase interacting multifunctional proteins (AIMP1-3). Recent studies show that bi-allelic mutations in AIMP2 cause a neurodevelopmental syndrome that overlaps phenotypically with disorders caused by recessive ARS mutations. In this work, we identify novel AIMP2 mutations (c.575-2A>G and c.75C>A) in a patient with a severe neurodevelopmental disorder characterized by microcephaly, cerebral atrophy, spasticity, and cognitive impairment, among other features. We show in patient derived fibroblasts that disease associated AIMP2 mutations cause loss-of-function at the level of protein expression, leading to attenuation of protein synthesis and dysregulation of the MSC component, methionyl-tRNA synthetase (MetRS). CRISPR-mediated disruption of aimp2 in zebrafish led to increased cell death and atrophy in the developing brain, further supporting a link between AIMP2 dysfunction and neurodevelopmental defects. To explore a method of enhancing AIMP2 levels, we tested the effect of anticodon engineered tRNAs on expression of disease-associated nonsense mutations and found that engineered tRNAs dramatically increase protein expression of AIMP2 variants. Together, these data demonstrate that AIMP2 depletion leads to neurodevelopmental dysfunction in both human and animal models, suggesting that AIMP2 stimulating therapies like anticodon engineered tRNAs could improve patient outcomes.

IT26

Development of a unique domain embedded in tRNA synthetase for cancer therapy

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Human aminoacyl-tRNA synthetases have acquired various unique domains during evolution to expand their roles beyond translation. Among them, a unique functional domain (UNE-C1) within the catalytic region of human cytosolic cysteinyl-tRNA synthetase (CARS1) was shown to work as an endogenous TLR2/6 agonist. The isolated UNE-C1 showed high thermostabilty and solubility and directly induced immunogenic death of cancer cells. Moreover, it activated antigen-presenting cells via TLR2/6 and enhanced the efficacy of cancer vaccines and immune checkpoint inhibitors, suggesting its potential as a novel immune-stimulating adjuvant.

IT27

Mammalian leucyl-tRNA synthetase from liver cancer cells modulates cellular senescence and autophagy

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Human cytoplasmic leucyl-tRNA synthetase (LARS) catalyzes the charging of tRNALeu with the amino acid leucine during translation to produce proteins. LARS is known as a nutrient sensor in an alternate activity to modulate cell growth. Because the aminoacyl-tRNA synthetases have been found to play a role in cancer, we hypothesized that LARS is a critical factor in cancer cell growth and tumor development. We found that LARS is over-expressed in human liver cancer and significantly, this corresponded in liver cancer human patients with challenged clinical outcomes. When LARS was knocked-down in HepG2 liver cancer cells, cell proliferation and growth was suppressed, yet cell migration was promoted without impacting global protein synthesis. Differentially expressed genes in response to LARS knockdown in HepG2 cells were localized. Moreover, autophagy with increasing autophagic flux was stimulated in HepG2 cells that had LARS knocked-down. Oxidative stress and decreased mitochondrial membrane potential was also induced with the depletion of LARS. Collectively, these results suggest that LARS is intertwined with the regulation of liver cancer cell proliferation via multiple cellular responses.

IT28

tRNA optimality as a determinant of cancer stemness in colorectal tumors

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Lgr5 colorectal cancer stem cells (CSCs) exhibit high plasticity and are critical drivers of tumor initiation, metastasis, and therapeutic resistance. Yet, the molecular mechanisms sustaining their functional identity remain not fully understood. Here, we identify tRNA optimality—regulated by dynamic tRNA pools, chemical modifications, and tRNA-modifying enzymes—as a central determinant of intestinal CSC maintenance.

Through integrated multi-omics profiling, we show that Wnt-driven transformation reprograms tRNA biology in Lgr5 CSCs, enhancing the translation of proteins required for stemness and plasticity. A functional CRISPR-Cas9 dropout screen in both normal and cancer-derived intestinal stem cells reveals that the tRNA methyltransferase Trdmt1 is selectively essential for Lgr5 CSC survival. Genetic ablation or pharmacologic inhibition of Trdmt1 impairs the translation efficiency of CSC- defining regulatory programs, thereby disrupting Lgr5 CSC-derived organoid formation and propagation.

Our findings uncover tRNA-mediated translational control as a key vulnerability in colorectal CSCs and nominate Trdmt1 as a selective therapeutic target to impair cancer stemness while sparing normal intestinal stem cells.

IT29

Therapeutic potential of an anti-KARS1 antibody blocking monocyte migration in PAH models

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Pulmonary arterial hypertension (PAH) is a devastating rare disease characterized by high mortality and severely impaired quality of life. A better understanding of its pathophysiology is essential for developing more effective treatments. Monocytes play a key role in the development and progression of PAH by recruiting inflammatory cells and promoting pulmonary vascular remodeling.

Here, we identified lysyl-tRNA synthetase 1 (KARS1) as a membrane protein specifically expressed on monocytes, where it plays a critical role in facilitating monocyte migration. We developed a human monoclonal antibody against KARS1 and demonstrated that it inhibits monocyte infiltration into the lung during acute lung injury and alleviates PAH symptoms *in vivo*.

BC-NKA-20008 (N3-8), an affinity-matured anti-KARS1 antibody, significantly reduced monocyte migration *in vitro* and extravasation *in vivo* by depleting membrane KARS1 through antibody-induced endocytosis. N3-8 was highly effective both as monotherapy and in combination therapy, increasing survival and significantly reversing right ventricular systolic pressure, right ventricular hypertrophy, fibrosis, and pulmonary vascular remodeling in animal models of PAH. Pro-inflammatory cytokines and chemokines were also significantly reduced following N3-8 treatment. Flow cytometry and immunohistochemistry analyses revealed elevated membrane KARS1 expression in monocytes/macrophages from both blood and lung tissues of PAH patients, suggesting a critical role for KARS1 in human PAH pathogenesis.

We propose that anti-KARS1 antibody represents a promising therapeutic candidate for PAH by targeting a novel mechanism of inflammation control to address the unmet need in current treatment strategies.

IT30

tRNA modifications: a new vulnerability driving cancer heterogeneity & tumor immune response

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Global regulation of mRNA translation has emerged as a central mechanism driving the adaptation of tumor cells during cancer progression and response to therapy. The emerging concept that translation reprogramming promotes the establishment of specific cancer phenotypes may position this important process as a key hallmark of cancer. Our previous work demonstrates that **tRNA epitranscriptomics** is a new regulatory module that contributes to the establishment of cancer phenotypes through the maintenance of specific proteomes. Therefore, modulation of tRNA epitranscriptomics and mRNA translation holds the potential to sustain tumor heterogeneity and shape tumor immune environment. Here, I will discuss how tRNA regulation machineries may represent new regulatory modules that support cancer phenotypes and dictate interaction of cancer cells with their immune environment. We aim to discover new vulnerabilities in cancer and to uncover original therapeutic opportunities to be exploited for the development of future anti-cancer treatment.

IT31

EPRS1 goes nuclear

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EPRS1 is a unique, bifunctional aminoacyl-tRNA synthetase (aaRS), and at 172 kDa is the largest constituent of the human multi-aminoacyl-tRNA synthetase complex (MSC). Noncanonical functions have been ascribed to EPRS1 following re-localization from the MSC to five different intracellular compartments. In the earliest known activity, EPRS1 joins three cytoplasmic proteins in monocyte/macrophages to form the GAIT complex that silences translation of inflammation-related mRNAs. A second anti-inflammatory activity is mediated by EPRS1 directing formation of an AKT-containing endosomal complex. In adipocytes, EPRS1 interacts with FATP1 at the plasma membrane to import long-chain fatty acids. In SARS-CoV-2-infected lung cells, EPRS1 joins three other MSC constituents at the endoplasmic reticulum to stimulate translation of virus sub-genomic RNAs. Recently, we reported the first nuclear function of EPRS1. In PTEN– breast cancer cells, EPRS1 is transported to the nucleus in an AKT-dependent manner. Mass spectrometric analysis identified PARP1 [poly(ADP-ribose) polymerase 1] as a major nuclear target. Nuclear EPRS1 activates ADP-ribosylation activity of PARP1, thereby inducing its principal function, namely, DNA damage-repair and consequent resistance to environmentally-induced DNA damage. Proteomic analysis of EPRS1-interacting proteins in the nucleus revealed an unrelated cancer-associated function of nuclear EPRS1 currently under investigation. The pro-tumorigenic role of EPRS1 is supported by patient studies that show elevated expression is associated with markedly reduced overall survival of patients with breast tumors, suggesting nuclear EPRS1 and its interactions are promising therapeutic targets.

Selected talks

ST1

Chiral discrimination and metal ion mediated translation quality control in alanyl-tRNA synthetase

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Error free synthesis of proteins is a prerequisite for their proper functioning, which is achieved through cellular proofreading activities. Many aminoacyl-tRNA synthetases mischarge D- enantiomer on to their cognate tRNA (Kuncha et al. *J Biol Chem*. 2019 16535-16548). Alanyl-tRNA synthetase (AlaRS) is a unique enzyme as its catalytic site can mischarge larger L-serine and smaller achiral glycine but not D-alanine. Our study shows that AlaRS catalytic pocket has conserved features responsible for rejecting D-alanine that supports the 'four-location' model proposed by Koshland. Thus, the catalytic site is designed for strict D-chiral rejection (Sivakumar et al. *Nucleic Acids Research* 2023 3327–3340). On the other hand, the universally conserved editing domain of AlaRS (AlaRS-Ed) is important as any defect in AlaRS editing can lead to severe pathologies. We show that an invariant Zn^{2+} binding motif in AlaRS editing domain possess tightly bound zinc that protects its activity from reactive oxygen species (ROS). In contrast, the homologous editing domain of ThrRS (ThrRS-Ed) lack zinc binding capacity and is affected by ROS leading to Thr-codon mistranslation. The study has revealed that a single residue is responsible for its functional divergence, which is rooted over ~3.5 billion years of evolution since LUCA (Gogoi et al. *Nat Commun.* 2025 10.1038/s41467-025-58787-2.

ST2

Decoding zinc domain dynamics and function in leucyl-tRNA synthetase

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Aminoacyl-tRNA synthetases (AaRS) are essential for high-fidelity translation of the genetic code, charging tRNAs with their cognate amino acids. Leucyl-tRNA synthetase (LeuRS), a class I AaRS, ensures accurate aminoacylation and proofreading of leucine onto $tRNA^{Leu}$ through distinct catalytic and editing domains. The zinc-binding (ZN) domain of *E. coli* LeuRS (*Ec*LeuRS) plays a dynamic role in stabilizing the tRNA during catalysis and facilitating CCA-end translocation during editing. While previous structures lacked a modeled ZN domain in the editing conformation, we report the first crystal structure of the *Ec*LeuRS–$tRNA^{Leu}$ complex in this state with the ZN domain fully resolved. Combined with site-directed mutagenesis and kinetic assays, our findings support a mechanistic model in which the ZN domain serves a dual function: initially gating the catalytic pocket to enable tRNA-independent amino acid activation, and subsequently repositioning to accommodate tRNA binding and stabilize its highly contorted 3' end during aminoacyl transfer while also possibly facilitating product release. These results deepen our understanding of LeuRS activities and have implication for the design of inhibitors targeting distinct functional states of this novel antibacterial target.

ST3

An openly available toolbox for the study of tRNA synthetases

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Aminoacyl-tRNA synthetases are essential and universally distributed enzymes that play a critical role in protein synthesis and are increasingly recognised as promising drug targets in antimalarial, antibacterial, and anticancer research.

OSM-S-106, a thienopyrimidine-based sulfonamide, has shown excellent potency, solubility, low toxicity, and favourable developability. Recent studies have demonstrated that OSM-S-106 targets *Plasmodium falciparum* asparaginyl-tRNA synthetase (*Pf*AsnRS) via a “reaction hijacking” mechanism, reacting with the tRNA–amino acid adduct to form a covalent OSM-S-106–asparagine conjugate (Wang et al., Nat. Commun. (2024) 15:937–937).

Building on this finding, we synthesised and evaluated the complete set of OSM-S-106 amino acid adducts, revealing a broad range of antiplasmodial activity. Notably, three compounds displayed low nanomolar potency in a standard blood stage assay, and five adducts tested showed on-target, selective inhibition of their respective tRNA synthetases. Among them, the OSM-S-106–proline adduct exhibited a Pf IC of 6 nM, no cytotoxicity against HepG2 cells and a binding affinity (K_d) to PfProRS below 1 nM. This compound is a potential chemical probe and drug candidate. Additionally, preliminary cross-screening of XRS Box compounds against Mycobacterium avium and E. coli has yielded encouraging results.

We propose that this set of 106 amino acid conjugates—termed the XRS box—represents a valuable and openly accessible toolbox for the study of tRNA synthetases in biology and drug discovery.

ST4

Coexisting tryptophanyl-tRNA synthetases endow a human microbial pathogen with distinct fitness advantages

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Numerous organisms across all domains of life encode more than one gene for a particular aminoacyl-tRNA synthetase (ARS). The “repeated” ARS genes generally display divergent phylogenetic origins and unique biochemical properties. Growing evidence suggests that organisms acquire additional ARS genes to sustain or adapt to their unique lifestyle. However, most cases of ARS repetition remain poorly understood. Here, we report the investigation of the two tryptophanyl-tRNA synthetase genes (TrpRS-A and TrpRS-B) encoded in the pathogenic microbe *Salmonella enterica*. We found that TrpRS-A is expressed constitutively to sustain protein synthesis under most growth conditions, whereas TrpRS-B is only expressed in specific environments. Despite their unique expression profiles, both TrpRS genes can be individually deleted, indicating that either TrpRS gene can sustain *S. enterica* and that a regulatory mechanism to compensate for the absence of each TrpRS exists. The single-gene TrpRS deletion strains exhibit distinct physiological characteristics, including slower growth in minimal media, increased intracellular survival in human macrophages, and sensitivity to the antimicrobial indolmycin. The deletion of the TrpRSs causes a large reprogramming of *S. enterica*’s transcriptome with the expression of several key genes differentially regulated. Biochemically, S. enterica TrpRSs show similar catalytic efficiencies for tRNA aminoacylation with Trp. However, they display distinct sensitivities to oxidative damage and specificities for tryptophan analogs. Collectively, these results indicate that the acquisition of two TrpRS genes in *S. enterica* is due to their distinct functional properties, and their synergy contributes to *S. enterica*’s fitness under specific conditions.

ST5

Phylogenetic reconstruction of ancestral coding alphabets using aaRS sequences

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All known living systems make proteins from the same twenty canonically-coded amino acids, but this was not always the case. Early genetic coding systems likely operated with a restricted pool of amino acid types and limited means to distinguish between them. Despite this, amino acid substitution models like LG and WAG all assume a constant coding alphabet over time. That makes them especially inappropriate for the aaRS. To address this limitation, we created a class of substitution models that have 20 amino acid types in the present day, and 19, 18, 17,... in the past. This was implemented as a Bayesian phylogenetic method, enabling this reducing-alphabet hypothesis to be tested. As expected, this hypothesis was strongly rejected by datasets composed exclusively of “young” eukaryotic proteins. It was generally supported by “old” (aaRS and non-aaRS) proteins whose origins date from before the last universal common ancestor. Standard methods overestimate the divergence ages of proteins that originated under reduced coding alphabets in both simulated and aaRS alignments. The new model reduces this bias substantially. Our findings support the late incorporation of tryptophan into the genetic code (relative to tyrosine) and suggest that isoleucine and valine were once coded interchangeably, forming protein quasispecies. This work provides a robust, seamless framework for reconstructing phylogenies and ancestral sequences for the aaRS and other proteins that evolved under reduced alphabets; and offers further insights into the early evolution of the aaRS.

Douglas et al., bioRxiv (2025) 2025-02.

ST6

A catalytic pathway to support prebiotic linkage between amino acids and RNA

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The aminoacyl ester bond is notoriously unstable. In cells, elongation factors shield the amino acid-tRNA linkage from hydrolysis. However, in the early stages of evolution—before such proteins existed—it remains unclear how this linkage was maintained. Here, we describe a prebiotically plausible catalytic context in which the attachment of amino acids to RNA can be greatly stabilised within a duplexed RNA system. We show that the fast, efficient, non-specific transfer of amino acids from the 2'/3' terminus of a templated oligonucleotide to a neighbouring 5' hydroxyl group offers a potential solution to this stability problem. We present a kinetic model for this system and show that this pathway occurs in tandem with prebiotically plausible aminoacylation chemistry, resulting in in situ activation and transfer, resulting in 5' aminoacylation, the most stable form of RNA esterification. This prosses provides a plausible route for the prebiotic accumulation of aminoacylated RNA, and we discuss contexts in which this could have led to peptide bond formation and, ultimately, the emergence of a primitive translation system.

ST7

Structural modularity, functional information, and the genesis of genetic coding

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Class I and II aminoacyl-tRNA synthetase (AARS) activate amino acids into a symbolic form for assembly. The co-linearity of gene and protein sequences is a familiar idea. Yet, few ask how it happened. That question has puzzled us. We try to model early AARS*tRNA cognate pairs experimentally to learn how they recognized both amino acid and RNA substrates. Short segments we call urzymes can chose amino acids from the correct Class and with the right properties about 9 times out of 10. Acylation of RNA minihelix libraries now shows that they also make comparable specific choices of RNA substrates (Carter et al., Genome Biol Evol. (2025) 17(6):evaf095). Ancestral AARS preferred minihelix substrates ten-fold over full-length tRNA (Tang et al., NAR (2024) 52(12):7096-7111). Complex amino acids at their active sites are not required for activity (Tang et al., NAR (2023) 51(15):8070-8084). These properties are unexpectedly common in sequence space. E. coli converts a double mutant LeuRS plasmid into a hierarchy of genes similar to the ~50-residue protozymes and ~120-130 residue urzymes we made to represent ancestral AARS. Two distinct ‘clades’ code for catalytically active synthetases. This striking in vivo result confirms our earlier work. AlphaFold3 predicts modular secondary structures for the LeuRS mRNA sequences coding for the simplest urzymes. Ancient AARS genes thus likely acquired code modules by a process akin to transposition, as proposed for ribosomal RNA. An active 81-residue variant has synthetase activities equal those of the 50% longer LeuAC urzyme. This variant also suggests how to design bidirectional genes for Class I and II AARS. Three very different connections between active-site signatures have about the same activity. A key implication is that the manifold of catalytically active ancestral AARS is far more inclusive than previously thought possible. The low functional information required to launch translation simplifies the problem of how the genetic code came to be.

ST8

Historical reconstruction of the glutamate-to-glutamine specificity switch in a resurrected ancient aminoacyl-tRNA synthetase

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Aminoacyl-tRNA synthetases set the universal genetic code by coupling each amino acid to its corresponding tRNA. How this essential machinery evolved has sparked intense interest that has led to a refined understanding of the biochemistry of extant synthetases. In parallel, phylogenetic studies have established that the ten or so synthetases in each of the two non-homologous classes evolved by a series of duplications from a common ancestor. Despite its potential for a mechanistic understanding of the evolution of the genetic code, combining synthetase biochemistry and phylogenetics has not been possible due to the high sequence divergence between synthetase paralogs. Here, we leverage the recent expansion of archaeal and eukaryotic taxon sampling to improve the Glu/GlnRS phylogeny, which enabled us to obtain well-supported ancestral reconstructed sequences for the nodes prior to and after the divergence of GluRS and GlnRS in early eukaryotes. Reconstructing the catalytic domain of AncGlxRS and AncGlnRS allowed us to map the historical substitutions that facilitated the specificity switch from glutamate to glutamine in the ancestral synthetases. In addition to the 4 substitutions reported in Bullock et al., PNAS (2008) 105:7428-7433, we identify 12 more historical substitutions that are putatively important for the specificity switch but were missed in the previous analysis that did not employ phylogenetics. To test the activity and specificity of the ancestral synthetases, we have developed a new non-radioactive assay that relies on the chemical capture of aminoacylated tRNA, which can be analyzed by gel or nanopore sequencing (White et al., bioRxiv (2024) 11.18.623114). Our work showcases the power of combining rigorous phylogenetics with biochemistry to study the evolution of synthetases and promises to let us peer into the biochemistry of even more ancient synthetases that shaped the evolution of the genetic code.

ST9

Proteomic profiling of isoleucine mistranslation in *Escherichia coli*

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The hallmark of protein biosynthesis is the high fidelity of the translation of genetic information. The faithful decoding of genetic information relies on the correct pairing between mRNA and aminoacylated tRNA, monitored by the ribosome, and the accuracy of coupling correct amino acids to tRNAs, governed by aminoacyl-tRNA synthetases.

Isoleucyl-tRNA synthetase (IleRS) faces a formidable challenge in discriminating structurally similar valine (Val) and norvaline (Nva) from cognate isoleucine (Ile) and relies heavily on its editing domain to maintain the high accuracy of $tRNA^{Ile}$ charging. We have engineered the *Escherichia coli* strain with disarmed IleRS, with inactive editing domain, and we have forcibly compromised the translational fidelity by supplementing the growth medium with excess Val or Nva. Up to 20 % of Val and Nva misincorporation was achieved in genetically engineered strain harboring editing-deficient IleRS, measured proteome-wide by shotgun proteomics. Proteomic profiling revealed upregulation of major chaperones, disaggregase ClpB and proteases, indicative of proteotoxic stress. Furthermore, excessive nonspecific protein aggregation, enriched in mistranslated polypeptides (close to 30 %) was observed, and bacterial growth, fitness and resistance to heat stress were reduced (Pranjic et al., Int. J. Biol. Macromol. (2024) 262:130068). Therefore, *E. coli* can sustain high levels of Val or Nva misincorporation, but it suffers dire pleiotropic consequences.

We have recently discovered that IleRS editing-deficient strain can accumulate ~15 % mistranslation under certain growth conditions, even when the growth medium is not supplemented with exogenous Val or Nva. This indicates that metabolic adaptation to different growth conditions can lead to unanticipated challenges to fidelity of protein synthesis. Changes in metabolic pathways jeopardizing the fidelity of protein biosynthesis will be deciphered by further proteomic analyses.

ST10

Drive genetic code expansion with a dual fluorescent reporter and FACS-based selection of functional aminoacyl-tRNA synthetases

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Expanding the genetic code with non-canonical amino acids (ncAAs) unlocks new possibilities in protein design for function. However, the discovery of aminoacyl-tRNA synthetase (aaRS) variants accepting structurally novel ncAAs as a substrate remains a challenge. We present a robust high-throughput platform combining a dual-fluorescent reporter system with fluorescence-activated cell sorting (FACS) to identify aaRS mutants from a gene library that enable the site-specific incorporation of structurally diverse ncAAs in *E. coli*.

The red-green dual fluorescence reporter is a fusion of monomeric Scarlet3 and monomeric EGFP containing an intervening in-frame amber stop codon. Green fluorescence appears only when the stop codon is suppressed by an ncAA or a canonical amino acid while the red fluorescence is independent of amber suppression. This system enables the relation of ncAA incorporation to gene expression as well as its normalization to the corresponding wild-type reporter. Inspection of the cells that are not supplemented with the ncAA facilitates the setting of the gate to sort those aaRS variants that accept the ncAA as a substrate. This omits a sorting step in the absence of the ncAA and simplifies sorting by clearly distinguishing true incorporation events from false positives.

To assess the platform's performance, we screened a *Methanomethylophilus alvus* PylRS (*Ma*PylRS) library containing five randomized substrate-binding residues against a selected set of ncAAs. These analogs had been previously shown to be incorporated by published *Ma*PylRS mutants and served as benchmarks to validate the system's reliability. Employing this screen, we isolated a *Ma*PylRS variant that accepts ^{OXO}SEAK, a lysine derivative featuring a highly reactive oxalyl thioester-based handle for bioconjugation (Snella et al., Angew. Chem. Int. Ed. (2022) 61:e202204992).

ST11

Engineering translation machinery for production of post-translationally modified selenoproteins in *E. coli*

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Post-translational modifications (PTMs) of amino acids are a key mechanism for regulating protein function in eukaryotes. Studying the role of these modifications in various biological processes requires functional studies in the presence and absence of these modifications. Advances in bacterial genetic code expansion (GCE) techniques have enabled site-specific insertion of noncanonical amino acids, including PTMs, into proteins. However, challenges arise when investigating the role of PTMs in selenoproteins. Selenoproteins, which contain selenocysteine, play essential roles in oxidative stress, immune response, and cell maintenance in humans. Unlike other proteins, overexpression strategies for selenoproteins are underdeveloped, requiring specialized protocols to efficient selenocysteine insertion. In this study, we combine our recently developed selenocysteine insertion technology with current GCE techniques to enable the overexpression of post-translationally modified selenoproteins in *Escherichia coli*. Our proof-of-principle experiments demonstrate the feasibility of this approach, and we have since optimized the efficiency of noncanonical amino acid insertion. This advancement allows for the study of each amino acid (selenocysteine or PTM) individually, as well as in combination, to better understand their roles in protein function.

ST12

Unknown role of arginyl-tRNA synthetase paralogue with a variation in the HIGH motif

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Key catalytic motifs are crucial for enzyme activity and therefore highly conserved. However, in some rare cases, their variations can provide enzymes with new traits and evolutionary advantages. In the case of class I aminoacyl-tRNA synthetases (AARS), HXGH (where X stands for any hydrophobic amino acid) and KMSKS serve as signature motifs participating in the amino acid activation via ATP binding in the ground and/or transition state. We have recently shown that natural variation of the HXGH motif provides isoleucyl-tRNA synthetases with extreme resistance towards the natural antibiotic mupirocin. This lends the idea that variations in the HXGH motif may be a proxy for relevant yet undiscovered AARS features.

In a predatory genus *Herpetosiphon*, we found a one-of-a-kind arginyl-tRNA synthetase (ArgRS) paralogue with a HVGH to RVDH variation. The first His in HXGH is crucial for transition state stabilization while Gly is vital for steric reasons, making the incorporation of Arg and Asp in their places intriguing. Proteomic analysis confirmed the expression of two ArgRS genes found in the genome, with the ArgRS-RVDH paralogue being expressed at a significantly lower level than the canonical ArgRS-HVGH. Kinetic characterization showed that the ArgRS-RVDH is inactive in both arginine activation and the two-step aminoacylation. Ligand binding analysis revealed that although this paralogue binds tRNA and Arg, it does not bind ATP. Exchange of the native RVDH motif into the canonical HVGH yields mutants still incapable of canonical ArgRS functions. As expected, mutation of the HVGH motif to RVDH in the aminoacylation-active canonical ArgRS inactivates the enzyme. Ongoing work is focused on testing whether the aminoacylation-inactive ArgRS paralogue modulates the activity of the canonical ArgRS via the formation of a binary complex. An intriguing hypothesis is that this atypical ArgRS with a varied HVGH motif plays some non-canonical role related to bacterial predation.

ST13

Expanding ribosomal catalysis via non-canonical monomers

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The ribosome, a natural biocatalyst, polymerizes L-α-amino acids into polypeptides by facilitating peptide bond formation through aminolysis within its peptidyl transferase center (PTC). Leveraging this unique catalytic environment, we explore the ribosome's potential to synthesize polymers featuring cyclic motifs in their backbones. To achieve this, we design various non-canonical monomers (ncMs) with bifunctional groups, which undergo ring-closing aminolysis reactions. Employing a cell-free translation system, we demonstrate the ribosome's ability to incorporate these ncMs sequentially into peptides, yielding unprecedented cyclic backbones. This work expands the chemical repertoire of ribosome-mediated biopolymer synthesis, paving the way for future advancements in engineered ribosomal chemistries for creating novel biomaterials.

ST14

Probing secretion mechanism and topology of extracellular glycyl-tRNA synthetase

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Secretion of aminoacyl-tRNA synthetases (aaRS) and their interaction with cell surface receptors have been recurrently reported and associated with human diseases. However, the secretion mechanism of aaRSs remains unclear. We previously showed that glycyl-tRNA-synthetase (GlyRS), associated with both neuromuscular disease Charcot-Marie-Tooth (CMT) and autoimmune antisynthetase syndrome (ASSD), is secreted from C2C12-differentiated myotubes (He et al., Nat. (2015) 526:710-714). The secretion is not affected by Brefeldin A, a classical secretory pathway inhibitor, but by a sphingomyelinase inhibitor, a blocker of both exosome biogenesis and autophagy. GlyRS is present in extracellular vesicles/particles (EV/EPs) isolated using differential ultracentrifugation. Compared with TSG101, an exosome marker protein, extracellular GlyRS is less protected from trypsin digestion, implying that the secreted GlyRS is either located on the exterior of vesicles or associated with membraneless particles/condensates, allowing for interaction with cell-surface receptors. Extracellular GlyRS exhibits altered epitope accessibility compared to recombinant protein, by differential recognition by monoclonal antibodies targeting distinct regions of the synthetase. Unconventional secretion pathways are often upregulated under stress. To investigate the secretion mechanism, we cultivated C2C12 mouse myoblasts under serum starvation and with hydrogen peroxide (“high stress”) or without hydrogen peroxide (“low stress”) treatment. Flow cytometry of EV/EPs revealed a $GlyRS^{+}/p62^{+}/\beta\text{-actin}^{+}$ subpopulation unique to “high stress” EV/EPs. Anti-WHEP domain antibody but not anti-dimerization interface antibody detected GlyRS in EV/EPs, hinting at GlyRS topology in EV/EPs. Western blot analysis show that the enrichment of GlyRS, β-actin, p62 and low LC3 I/II ratio in “high stress” versus “low stress EV/EPs, indicating secretory autophagy as a major pathway for the secretion of GlyRS under stress.

ST15

The tRNA multisynthetase complex is critical for the cardiac response to stress

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Understanding how the translation machinery contributes to cellular adaptation is critical for decoding tissue-specific responses to stress. In the heart, an organ that is highly dependent on precise protein homeostasis, this question is particularly critical, as its capacity to withstand injury determines overall survival. Despite the central role of gene expression regulation in cardiac disease, the specific contribution of the protein synthesis machinery, especially aminoacyl-tRNA synthetases (ARSs) and their interacting partners, remains largely understudied. Our research focuses on the multisynthetase complex (MSC), an assembly of multiple ARss with auxiliary scaffold proteins (AIMP1–3) to support translation and coordinate stress responses. We identified AIMP2 as a key structural and regulatory component within the MSC in the cardiac system. Specifically, we observed a significant reduction in AIMP2 levels in failing hearts, suggesting its involvement in the heart's adaptive response to stress.Using a cardiomyocyte-specific AIMP2 knockout mouse model, we demonstrate that loss of AIMP2 leads to pathological cardiac remodeling and impaired function. Mechanistically, AIMP2 deficiency disrupts the stability of MSC components, including glutamyl-prolyl-tRNA synthetase (EPRS) and Lysyl-tRNA synthetase (KARS), contributing to impaired translational control under stress conditions. Together, our findings so far reveal a previously unappreciated role for AIMP2 in maintaining the integrity of the MSC and cardiomyocyte homeostasis during stress. These insights expand the frontier of ARS biology by linking translation machinery dynamics to tissue-specific stress responses in vivo.

ST16

Investigating the scope of thioester-mediated aminoacylation of RNA with non-canonical amino acids

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Aminoacyl-tRNA synthetases (AARSs) and flexizyme ribozymes have enabled the attachment of a wide range of non-canonical amino acids to RNA. However, both systems can face challenges when working with substrates that are especially bulky or functionally dense. Alternative chemical strategies may therefore support broader substrate incorporation and offer complementary tools for RNA aminoacylation.

We are developing a thioester-mediated approach for site-selective 2′,3′-aminoacylation of RNA oligonucleotides under mild aqueous conditions, specifically for non- canonical amino acids. This method builds on recent work from the Powner laboratory showing that α-amino thioesters can achieve regioselective terminal acylation of RNA (Singh et al., ChemRxiv (2024), doi:10.26434/chemrxiv-2024-kbkvg). We are applying this chemistry to amino acids with physicochemical properties that are typically less compatible with enzymatic or ribozymic aminoacylation. We will discuss the key parameters underlying this chemistry.

Investigating the underlying chemical reactivity and mechanistic features of thioester- based coupling to RNA could provide a platform to expand the accessible substrate scope of RNA aminoacylation. We aim to define the conditions, limitations, and selectivity of this process to support its use in genetic code expansion and synthetic translation systems as a useful complement to AARS- and flexizyme-based strategies in the development of novel translational frameworks.

ST17

Virus-assisted directed evolution of nonsense suppressor tRNAs in mammalian cells

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Genetic code expansion technology enables the site-specific incorporation of noncanonical amino acids (ncAAs) with unique chemistries into proteins expressed in living cells. It uses engineered aminoacyl-tRNA synthetase (aaRS)/tRNA pairs, orthogonal in the host cell, to co-translationally incorporate ncAAs in response to nonsense or frameshift codons. However, its application in mammalian cells has been limited in scope by the suboptimal performance of the nonsense suppressor tRNA since these heterologous tRNAs don't efficiently interface with the foreign translation machinery of the host. While directed evolution could potentially address this limitation, implementing it in mammalian cells has proven challenging. We have developed a novel directed evolution strategy that couples the performance of the suppressor tRNAs to the proliferation of a mammalian virus. This approach allows for directed evolution and deep mutational profiling of these tRNAs directly in mammalian cells, yielding more efficient mutants. We found that such improvements in tRNA activity are often host cell-specific, indicating that the associated mutations likely allow the engineered tRNAs to interface more efficiently with the unique translational apparatus of the mammalian host. The improved ncAA incorporation efficiencies offered by our enhanced suppressor tRNAs have broadened the scope of previously challenging applications, such as the incorporation of multiple distinct ncAAs per protein and facile development of mammalian cells stably expressing the ncAA incorporation machinery. Finally, we show that our virus-assisted directed evolution strategy can be extended to additional targets, including human-derived tRNAs, which are attractive candidates for nonsense suppression therapy for treating genetic disorders involving premature termination codons in important genes.

ST18

Drosophila models of histidyl-tRNA synthetase-associated peripheral neuropathy

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Dominantly inherited mutations in 8 tRNA synthetase genes cause Charcot-Marie-Tooth disease (CMT), a peripheral neuropathy characterized by degeneration of motor and sensory axons. Overexpression of $tRNA^{Gly}$ rescued peripheral neuropathy defects in mouse and Drosophila models of CMT caused by mutations in glycyl-tRNA synthetase (GlyRS), supporting a mechanism by which CMT-mutant GlyRS sequesters $tRNA^{Gly}$, depleting the cellular pool of charged $tRNA^{Gly}$ leading to ribosome stalling and activation of the integrated stress response (Mora et al., NAR (2025) 53, Spaulding et al., Science (2021) 373:1156–1161, Zuko et al., Science (2021) 373:1161-1166). Unlike GlyRS, CMT-mutations in histidyl-tRNA synthetase (HisRS) have not been extensively characterized. Using *Drosophila*, we set out to evaluate whether a gain-of-toxic function or a loss-of-function mechanism underlies CMT-HisRS. We employed three different approaches: (i) overexpression of wild- type (WT) or mutant human HisRS (hHisRS), (ii) knock-down of the Drosophila endogenous HisRS (dHisRS) and (iii) knock-in of disease-causing mutations in dHisRS. Although mutant and WT hHisRS proteins were stably expressed, overexpression did not induce phenotypes. RNAi-mediated knockdown induced CMT- related phenotypes, including shortened lifespan, motor deficits and inhibition of protein synthesis. In our knock-in models, hemizygous males were lethal (the dHisRS gene is located on the X-chromosome), and we could rescue lethality by overexpressing WT HisRS but not $tRNA^{His}$. Altogether, these data suggest that HisRS mutations do not cause disease through a gain-of-toxic-function mechanism. Our data also argue against a dominant negative mechanism, with the caveat that hHisRS may not or less efficiently dimerize with endogenous dHisRS than it does with WT hHisRS. Therefore, to formally exclude a dominant negative mechanism underlying CMT-HisRS, we are currently evaluating the effect of overexpression of WT or CMT-mutant dHisRS.

ST19

Engineered LysRS switches: uncoupling translation from Ap4A signaling with therapeutic implications

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Lysyl-tRNA synthetase (LysRS, encoded by KARS) is an essential aminoacyl-tRNA synthetase that charges lysine onto its cognate $tRNA^{Lys}$ for protein synthesis. In addition to this canonical role in translation, human LysRS moonlights as a catalyst for the synthesis of diadenosine tetraphosphate (Ap4A), a nucleotide second messenger involved in diverse cellular signaling pathways. Notably, Ap4A produced by LysRS modulates immune responses; for example, it triggers transcriptional activation in mast cells and attenuates cGAS–STING-mediated interferon signaling. This dual functionality situates LysRS as a key node linking amino acid metabolism to signal transduction. However, understanding and selectively dissecting these two activities has remained challenging, given that both occur at the same enzyme active site.

Here we report a structure guided strategy to functionally segregate LysRS's activities. Using complementary quantum and classical mechanics simulations, we designed enzyme variants that act like catalytic microswitches by disrupting one specific activity while preserving the other. Biochemical and kinetic characterization of these LysRS mutants confirms that the canonical tRNA-charging and moonlighting Ap4A-synthesizing functions can be uncoupled without compromising the essential common structural requirements. Selective modulation of LysRS's dual roles offers a new approach to probe the biological significance of Ap4A signaling in cells without perturbing translation. Such tailored LysRS variants enable us to delineate the contribution of Ap4A to physiological and pathological processes (e.g., inflammatory and oncogenic pathways) independently of protein synthesis. In addition, we analyzed pathologic mutations affecting synthetase activity in a pediatric patient to characterize the effects of the substitutions and present a novel potential therapeutic approach.

ST20

tRNA and amino acid supplementation rescues mistranslation caused by pathogenic HARS alleles

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Pathogenic variants in aminoacyl-tRNA synthetases cause a variety of severe and extremely rare genetic conditions, for which little or no disease-specific treatments yet exist. Autosomal dominant mutations in histidyl-tRNA synthetase (HARS) cause the genetic disorder Charcot-Marie-Tooth type 2W (CMT2W). Treatments are currently restricted to symptom relief, with no therapeutic available that targets the cause of disease. We elucidated the molecular and cellular basis for several pathogenic HARS alleles, identifying HARS insufficiency, thermal instability and toxic gain of functions for diverse alleles. We found that HARS toxic gain of function can be alleviated with histidine supplementation in some cases, while other alleles required cognate tRNA supplementation to rescue mistranslation. Finally, for alleles not rescued by either amino acid or tRNA substrate, we tested small molecule inhibitors to treat cellular effects of pathogenic HARS alleles.

ST21

Leucyl-tRNA synthetase as a therapeutic target for Duchenne muscular dystrophy

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Progressive muscle weakness and deterioration of the muscular system result in early death of patients of Duchenne muscular dystrophy (DMD), the most common and severe form of muscular dystrophies diagnosed in childhood. Currently there is no cure or satisfactory therapy for this devastating disease. Impaired autophagy is one of the cellular hallmarks of DMD, contributing to the disease progression. Molecular mechanisms underlying the impairment of autophagy in DMD are not well understood. We find that leucyl-tRNA synthetase (LeuRS) is overexpressed in the muscles of *mdx* mouse, a model for DMD. Analysis of single-nucleus RNA sequencing data reveals that LeuRS expression is elevated in muscle stem cells of both *mdx* mice and DMD patients, and this is not a common expression pattern for other AARSs. Our investigation has further uncovered a non-translational function of LeuRS upstream of mTORC1 in suppressing autophagy in the dystrophic muscle. We find the specific inhibitor of RagD-LeuRS interaction, BC-LI-0186, to ameliorate muscle damage, reduce pathological fibrosis, increase muscle size, promote muscle force production and enhance overall muscle strength in the *mdx* mice. Furthermore, we show that these muscle improvements are due to restoration of autophagy. Interestingly, despite LeuRS acting through mTORC1, the mTORC1 inhibitor rapamycin does not have the same beneficial effects as BC-LI-0186 on dystrophic muscles. Taken together, our findings suggest that LeuRS is a unique and promising therapeutic target, which would represent the first example of a house-keeping protein being targeted therapeutically in DMD.

ST22

Hijacking aminoacyl-tRNA synthetases: a new strategy for development of anti-infectives

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In an effort to battle increasing levels of resistance of malarial parasites to current drugs, we screened a series of nucleoside sulfamates from a Takeda Pharmaceuticals library and identified compounds that exhibit high potency, high selectivity and long half-lives *in vivo*. An exemplar compound, ML471, exhibits single dose oral efficacy in a humanised mouse model of malaria. We identified the target as *P. falciparum* tyrosyl-tRNA synthetase (*Pf*Tyr-RS). *Pf*Tyr-RS catalyses the formation of a highly stable inhibitory sulfamate conjugate, via an unexpected mechanism, which we call reaction-hijacking (Xie et al., Science (2022) 376:1074-1079, Xie et al., PLoS Pathog (2024) 20:e1012429). This was the first example of aaRS targeting via this novel mechanism. Enzyme kinetics and X-ray crystallographic studies of plasmodium and human Tyr-RS reveal differential flexibility of a loop over the catalytic site that is associated with differential susceptibility to hijacking by nucleoside sulfamates (Xie et al., Science (2022) 376:1074-1079, Xie et al., PLoS Pathog (2024) 20:e1012429).

We also explored pyrimidine benzene sulphonamides, based on a hit from a GSK Library. An exemplar, OSM-S-106, exhibits drug-like physical parameters and a synthetically accessible scaffold (Xie et al., Nat Commun (2024) 15:937). It shows potent activity against parasite cultures and low mammalian cell toxicity. Again, we revealed a reaction hijacking mechanism and identified the target as the *P. falciparum* cytoplasmic asparagine RS (*Pf*AsnRS). Targeted mass spectrometry revealed that *Pf*AsnRS, but not human AsnRS, transforms the pro-inhibitor into an inhibitory conjugate. X-ray crystallographic and molecular modelling studies of *P. falciparum* and human AsnRSs in complex with the Asn-OSM-S-106 adduct

revealed features that underpin potency and selectivity (Xie et al., Nat Commun (2024) 15:937).

We are currently exploring the design of bespoke nucleoside sulfamates with tuneable specificity that target different aaRSs, for applications in malaria and bacterial infections.

ST23

Nanopore sequencing of intact aminoacylated tRNAs

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Transfer RNAs (tRNA) are decorated during biogenesis with a variety of modifications that modulate their stability, aminoacylation, and decoding potential during translation. The complex landscape of tRNA modification presents significant analysis challenges and to date no single approach enables the simultaneous measurement of important but disparate chemical properties of individual, mature tRNA molecules. We developed a new, integrated approach to analyze the sequence, modification, and aminoacylation state of tRNA molecules in a high throughput nanopore sequencing experiment, leveraging a chemical ligation that embeds the charged amino acid in an adapted tRNA molecule. During nanopore sequencing, the embedded amino acid generates unique distortions in ionic current and translocation speed, enabling application of machine learning approaches to classify charging status and amino acid identity. Specific applications of the method indicate it will be broadly useful for examining relationships and dependencies between tRNA sequence, modification, and aminoacylation. We will discuss our efforts to apply aa-tRNA-seq to study misaminoacylation in cytosolic and mitochondrial tRNA populations.

ST24

Predictive modeling to define the locus heterogeneity of tRNA synthetase-related peripheral neuropathy

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Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed, essential enzymes that ligate amino acids to tRNAs. Variants in seven cytoplasmic ARS genes cause dominant axonal peripheral neuropathy, presenting the question: how do variants in cytoplasmic ARSs lead to phenotypes restricted to the peripheral nervous system? While defects in protein translation and activation of the integrated stress response have been implicated downstream of certain neuropathy-associated ARS variants (Spaulding et al., Science (2021) 373:1156-1161), a unifying pathological mechanism that explains the locus heterogeneity has not been identified. All seven neuropathy-associated ARSs function as dimers and most pathogenic alleles are loss-of-function missense variants that do not affect protein levels, consistent with a dominant-negative effect.

If a dominant-negative effect is the disease mechanism, then variants in any dimeric, cytoplasmic ARS could exert a dominant-negative effect and lead to dominant neuropathy. To test this, we engineered missense mutations predicted to affect catalytic function in threonyl-tRNA synthetase (*TARS1*), a dimeric, cytoplasmic ARS not implicated in neuropathy. Of the variants tested, five are loss-of-function alleles in a humanized yeast complementation assay and two of these alleles repress wild-type *TARS1* in a humanized yeast dominant toxicity assay. The allele with the strongest effect, H590A, was previously reported as dominantly lethal in *E. coli* (Sankaranarayanan et al., Cell (1999) 97:371–381). We introduced this allele into the endogenous worm *tars-1* locus using CRISPR-Cas9. We have confirmed that it is a loss-of-function allele and are testing heterozygous worms for dominant neurologic and motor behavior phenotypes. We also generated a mouse line heterozygous for this allele and will test these animals for neuropathic phenotypes in behavioral and histopathological assays. Here, I will present all of our unpublished data from this study.

ST25

From mechanism to optimized inhibitors with enhanced selectivity targeting threonyl-tRNA synthetase

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Aminoacyl-tRNA synthetases (aaRSs) represent a promising yet underexploited class of therapeutic targets for antimicrobial and anticancer drug development. Our integrated structural, biochemical, and medicinal chemistry studies on threonyl-tRNA synthetase (ThrRS) have yielded fundamental mechanistic insights and practical drug discovery outcomes. We first identified obafluorin (OB) as a novel covalent inhibitor that targets Tyr462 in the ThrRS active site through β-lactone ring opening, aided by zinc ion coordination. This work revealed the unexpected capability of β-lactones to modify not only tyrosine but also lysine and arginine residues, expanding the scope of covalent inhibitor design. Subsequent investigations uncovered a critical role of Gly463 in governing ThrRS conformational dynamics, where mutations (G463S/G463A) conferred resistance by restricting active site flexibility while maintaining catalytic function - explaining the self-resistance mechanism in OB-producing *Pseudomonas fluorescens*. Building on these mechanistic foundations, we have successfully developed optimized OB derivatives through structure-based design, achieving improved selectivity for bacterial versus human ThrRS orthologs. Our findings provide a comprehensive framework for developing next-generation aaRS inhibitors that combine covalent targeting strategies with conformational dynamics considerations and species-selective optimization. This work bridges fundamental understanding of enzyme inhibition mechanisms with practical applications in drug discovery, offering novel insights for targeting aminoacyl-tRNA synthetases.

ST26

Mechanisms and therapeutic applications of transfer RNA

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Protein truncating variants caused by premature termination codons (PTCs) are the most prevalent class of rare mutations in genetic disorders. Since these PTC mutations are individually rare and occur throughout the coding region of a gene, they represent a significant therapeutic challenge. We are developing engineered transfer RNA to repair such truncating variants. Data will be presented from a high-throughput screen of stop codon-rescuing human tRNAs with activity in human cells for the rescue of PTC codons. This library represents all human tRNA genes engineered for activity against all three stop codons. For any potential read-through therapy, it is essential to assess differential suppression of PTC and native stop codons. For this purpose, we have developed a proteomics method and bioinformatics pipeline to quantify encoding events at native stop codons. These data show minimal interactions between rescue tRNAs with native stop codons, an outcome consistent with previous ribosome profiling data, and suggest that a 'stop codon bias' exists between PTCs and native stop codons. Further, we find that in vitro PTC rescue is observed across multiple Arg-TGA patient PTCs within CDKL5, a neuronal gene with significant PTC burden. We also find that the co-expression of a rescue tRNA over-rides the nonsense mediated mRNA decay pathway of a PTC containing mRNA, leading to increased mRNA stability and full-length protein expression. Lastly, efficient viral delivery of tRNA payloads has been achieved in iPSC cells, and murine brain and retina to demonstrate tolerated rescue activity and durable expression of rescued protein. These viral vectors have been used to restore function to a W53X PTC in the Kir7.1 potassium channel and vision in a related model of murine Leber Congenital Amaurosis. In composite, the data support the continued development of engineered tRNA for the rescue of premature termination codons that cause rare genetic disease.

ST27

Temperature sensitivity of aminoacyl-tRNA synthetase variants and its implications for disease

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Fever is a common physiological response to viral infections, particularly during infancy, and is typically associated with enhanced immune function. However, in a subset of pediatric patients, febrile episodes coincide with neurodevelopmental regression, wherein previously acquired skills are lost. While some children recover these skills, others experience persistent developmental stagnation. Such clinical presentations often lead to the identification of underlying genetic etiologies.

In this study, we report on five patients harboring variants in genes encoding aminoacyl-tRNA synthetases (aaRSs), specifically *GARS1*, *YARS1*, *LARS1*, and *NARS1*, identified through whole-exome sequencing. Initial functional assays in patient-derived fibroblasts revealed near-normal aminoacylation activity under basal conditions. However, when assays were conducted under stress conditions—specifically, lysate pre-incubation at 40°C and reduced amino acid substrate concentration—a marked reduction in aminoacylation was observed. In some cases, this temperature-sensitive enzymatic impairment correlated with febrile episodes and clinical regression, as documented by the attending physician.

To investigate the molecular mechanism, we expressed and purified six missense variants (GlyRS: 2, TyrRS: 3, AsnRS: 1) in prokaryotic system. Thermal shift assays demonstrated that four variants exhibited significantly reduced protein stability, with melting temperatures decreased by up to 8°C relative to wild-type. These findings

suggest that the pathogenic variants may confer a temperature-sensitive loss of function due to conformational instability, potentially exacerbated during febrile episodes. Our findings support the inclusion of stress-based functional assays in routine diagnostics for aaRS-related disorders. Further research is needed to assess whether temperature sensitivity is a broader feature of aaRS variants and their implications for fever management in genetically susceptible individuals.

ST28

Elucidating the role of human multi-aminoacyl-tRNA synthetase complex (MSC) in hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL)

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Hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL) is a rare neurodevelopmental disorder associated with mutations in the DARS gene, which encodes aspartyl-tRNA synthetase (DARS), an essential component of the multi-aminoacyl-tRNA synthetase complex (MSC). While MSC is traditionally associated with protein synthesis, recent evidence implicates DARS in nuclear processes critical for regulatory functions beyond translation.

In this study, we uncover novel nuclear interactions between DARS and the splicing factor SRRM2 and chromatin organizer SATB2, confirmed through immunoprecipitation and co-immunoprecipitation assays. These interactions suggest an expanded role for DARS in nuclear regulation, potentially disrupted in HBSL. Using oligodendrocyte-enriched brain organoids derived from HBSL models, we identified significant morphological alterations in cortical neurons, most notably a reduction in SATB2-positive cells. This observation, reinforced by immunohistochemistry and single-cell RNA sequencing, highlights gene expression alterations related to spliceosomal activity and white matter integrity.

Our findings establish that DARS interactions with SRRM2 and SATB2 are essential for maintaining nuclear functions, particularly in splicing regulation and chromatin organization. Disruption of these nuclear pathways in HBSL likely underpins abnormal neurodevelopment and myelination deficits characteristic of the disease. This study offers a novel perspective on the nuclear roles of DARS, providing critical insights into the molecular mechanisms driving HBSL pathogenesis.

Poster abstracts

P1

Engineered mistranslating tRNAs to suppress huntingtin production and aggregation in cellular models of Huntington's disease

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Huntington's disease (HD) is a progressive neurodegenerative disorder caused by expanded CAG repeats in the *HTT* gene, resulting in huntingtin (HTT) proteins with abnormally long polyglutamine (polyQ) tracts. Many emerging treatments focus on reducing *HTT* expression or preventing polyQ aggregation, e.g., antisense oligonucleotides. Previous work showed that encoding non-glutamine residues into polyQ sequences can lessen aggregation and toxicity. Engineered transfer RNAs (tRNAs) enable targeted mistranslation, allowing site-specific amino acid substitutions to interrupt harmful polyQ expansions. Since certain aminoacyl-tRNA synthetases, including alanyl- and seryl-tRNA synthetase, do not rely on anticodon recognition, these tRNAs can be reprogrammed to decode glutamine codons (CAG). We demonstrated one such glutamine-decoding $tRNA^{Ala}$ variant that significantly reduced insoluble mutant HTT aggregates in ectopic models of HD (Tennakoon et al., Mol. Ther. Nucleic Acids (2025) 36:102442), as well as a glutamine-decoding $tRNA^{Ser}$ variant which lowered both mutant HTT production and aggregation. We are now expanding these approaches to more clinically relevant cell systems, including a PC12 cell line with inducible, genome-encoded *HTT* exon 1 expansions and patient-derived induced pluripotent stem cell (iPSC) neurons. These proof-of-concept studies will assess whether mistranslating tRNAs can reduce aggregate burden and mutant HTT toxicity under conditions more closely recapitulating HD phenotypes. By confirming the translational potential of missense suppressor tRNAs, we aim to develop new and innovative therapeutic approaches for HD and other polyQ-related disorders.

P2

In vitro exploration of the multi-functional *Mycoplasma penetrans metS* gene product

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It is known that the *metS* gene of *Mycoplasma penetrans* (Mp) codes for a multi-functional methionyl-tRNA synthetase (MetRS) that is double the size of other bacterial homologs. MpMetRS has at least two known active sites, one that produces methionine from the alpha keto precursor 2-keto-4-methylthiobutyrate (KMTB) in an aminotransferase domain (ATD) and another that attaches methionine to $tRNA^{Met}$ in an aminoacylation domain ($MetRS_{core}$). The ATD of MpMetRS contributes the only aminotransferase activity encoded in the *M. penetrans* genome. This leads us to question whether a need for methionine in the $MetRS_{core}$ of the protein signals the ATD to produce methionine, and ultimately how that methionine is delivered to the aminoacylation domain. Here we investigate whether methionine production increases in the presence of excess uncharged $tRNA^{Met}$, which would suggest that uncharged $tRNA^{Met}$ serves as a signal for enhanced aminotransferase activity. The ability of methionine to travel through a pathway that connects these two domains will also be studied. Co-expression and dual affinity chromatography will be used to isolate a heterodimeric protein, where half of each monomer is catalytically defective. The heterodimeric complex will be studied using aminoacylation and aminotransferase assays, to determine if a channeling pathway for methionine is feasible. The long-term goal of this project is to understand the cellular significance of the multi-functional product of the *M. penetrans metS* gene.

P3

Translation perturbance in tRNA synthetase-defective trichothiodystrophy

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Trichothiodystrophy (TTD) is a multisystem disorder characterized by skin, neurological, and growth abnormalities. So far, the disease has been associated with mutations in eleven genes involved in different biological processes. Among them, four genes encoding aminoacyl-tRNA synthetases (aaRSs), namely cysteinyl-, threonyl-, methionyl-, and alanyl-tRNA synthetases (CARS1, TARS1, MARS1 and AARS1). Mutations resulting in TTD cause a decrease in the amount of the corresponding aaRS and in efficiency of aminoacylation rate in patient primary fibroblasts. To assess the quality of translation, we have now investigated the translation error rate in MARS1, TARS1 and AARS1-defective TTD cells by using a a luciferase-based assay. We found a high translation error rate in all tested TTD cells, indicating translation infidelity. In addition, we addressed the question whether aaRS defects in TTD cells would affect accuracy of tRNA charging. By amino acid sensitivity assays, we found hypersensitivity to specific non-cognate aminoacids, suggesting that TTD-related variants likely impair the charging accuracy of the encoded enzymes. These defects were accompanied by accumulation of misfolded proteins in patient's fibroblasts, showing a loss of proteostasis. Thus, translation errors and consequent disrupted proteostasis emerge as a pathogenic mechanism, potentially contributing to developmental defects and neurodegeneration in TTD patients.

P4

Defining molecular mechanisms and developing treatments for AARS2-related mitochondrial disease

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Pathogenic biallelic variants in alanyl-transfer RNA (tRNA) synthetase 2 (AARS2)-cause a rare and progressive disorder typically characterized by infantile-onset cardiomyopathy and neurodegeneration with or without leukoencephalopathy, leading to a constellation of symptoms. There is no cure, but a deep understanding of pathological mechanisms will benefit efforts to develop effective therapeutics. Supplementation with cognate amino acids to alleviate bottlenecks in mitochondrial protein translation has been suggested as a promising treatment approach to rescue dysfunctional tRNA synthetases. Emerging evidence suggests AARS2 has additional non- canonical functions beyond translation, and these need to be defined in disease and considered when developing treatments. We will use biotin proximity labelling (Bio-ID) in HEK293T cells to identify which proteins wild type and variant AARS2 interact with, before exploring whether alanine supplementation can improve the canonical and non-canonical functions of patient AARS2 variants. Biotinylated proteins will be identified using LC-MS/MS and their interaction strength will be inferred based on peptide spectral counts and enrichment levels. Top interactions will be validated and further explored using patient fibroblasts and AARS2 knockout HEK293T cells. This work will uncover new functions of AARS2, and define how pathogenic AARS2 variants affect these. Access to patient-derived induced pluripotent stem cells (iPSCs) will also support investigations. These insights could support the development of supplementation-based therapeutic strategies for AARS2-related mitochondrial disease and identify new targets for treatment development.

P5

From mitochondrial translation to cell cycle: unveiling a novel regulatory mechanism

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SLIMP, a Mitochondrial Seryl-tRNA synthetase like protein, was described as a multifunctional protein originated from a duplication of the mitochondrial seryl-tRNA synthetase (SerRS2), early in animal evolution. These two proteins form a heterodimeric structure, essential for their aminoacylation function in Drosophila melanogaster mitochondria. Moreover, SLIMP interacts with LON protease, being essential for the control of mitochondrial DNA (mtDNA) copy number. Others have shown that an in vivo co-depletion of SLIMP and E2F1, a major regulator of cell cycle, is able to rescue cell viability, however, the involvement of SLIMP in this pathway is still not well understood.

Here we describe a novel non-canonical function of SLIMP as a link between cell cycle progression and mitochondrial dynamics. Upon in vitro and in vivo knockdown of this protein, we observe a premature entry in S-phase with accumulation of cells in G2. Interestingly, a rescue of SLIMP without the mitochondrial signal peptide (MSP) was able to reverse the G2 accumulation phenotype, suggesting an extra mitochondrial function of SLIMP. In mitochondria, SLIMP depletion lead to an increase in LON levels. This enables LON to cleave OPA1, an essential protein for mitochondrial fusion that, when cleaved, induces fission. We propose that, during G1, SLIMP localizes to the mitochondria where its levels allow for maintenance of mtDNA and fusion of mitochondria (essential for G1). During S phase and G2, SLIMP exits the mitochondria, allowing mtDNA synthesis and mitochondrial fission through cleavage of OPA1. Outside of the mitochondria, SLIMP regulates the expression of genes essential for DNA replication, allowing for a proper S-phase entry and exit.

To our knowledge, this is the first description of a protein that links and coordinates cell cycle progression and mitochondrial dynamics. We believe that our work provides new insight into understanding the importance of this organelle in cell cycle regulation.

P6

Inactivation of Maf1, global negative regulator of tRNA transcription, leads to defects in translation elongation in yeast

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Ribosomes decode mRNAs one codon at a time, and the efficiency of this process is heavily influenced by the availability of aminoacylated tRNAs. In both yeast and mammals, tRNA levels are regulated by the highly conserved protein Maf1. Maf1 controls tRNA synthesis by directly regulating RNA polymerase III transcription, and perturbations to Maf1-mediated regulation are expected to have a downstream impact on mRNA translation. In this study, we used a combination of classical yeast genetics and high-throughput sequencing to determine the effects of Maf1 inactivation on mRNA translation in yeast. In a genetic screen, we identified the translation elongation factor eEF1A as a suppressor of the maf1Δ growth defect. Protein synthesis was decreased in the absence of Maf1, but this phenotype was partially reversed upon eEF1A overexpression. Ribosome profiling revealed increased ribosome density across nearly all mRNAs, suggesting globally slowed translation elongation in maf1Δ cells. Indeed, tRNA abundance was significantly altered in the absence of Maf1, and tRNA species with decreased expression showed increased ribosome pausing over the cognate codon. Finally, Maf1 deletion led to increased translation of genes involved in amino acid biosynthesis. We conclude that Maf1- mediated regulation of tRNA expression is a critical determinant of global translation.

P7

Structural basis for leucyl-tRNA synthetase assembly into the human multi-tRNA synthetase complex by isoleucyl-tRNA synthetase

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Aminoacyl-tRNA synthetases are essential enzymes responsible for loading tRNAs with their correct amino acids. In mammals, eight AaRSs and three accessory proteins assemble into the Multi-tRNA Synthetase Complex (MSC). Structurally, the MSC assembles around the tetramerisation of glutathione transferase (GST) homology domains, which act as a platform for the assembly of other domains or proteins. Lysyl-, arginyl- and glutaminyl-tRNA synthetases assemble to the GST core via the auxiliary proteins AIMP1 and AIMP2, while asparaginyl-, methionyl and glutamyl-prolyl-tRNA synthetases assemble independently on the core. The isoleucyl-tRNA synthetase (IleRS) assembles indirectly, via an interaction between its UNE-I domain and the glutamyl-prolyl-tRNA synthetase. The leucyl-tRNA synthetase was shown to interact with IleRS; however, the molecular mechanism of this interaction was unknown. Here we uncover the molecular details of the interaction between human isoleucyl-tRNA synthetase and leucyl-tRNA synthetase. Our cryo-electron microscopy data suggest a flexible attachment between the catalytic cores of the two synthetases, with the UNE-L domain of LeuRS being key for the interaction with IleRS. Our results provide the structural basis for the assembly of LeuRS into the MSC via its interaction with IleRS. The LeuRS-IleRS interaction interface is far from the tRNA binding regions, and the observed flexibility corroborates with previous works suggesting a more flexible assembly for the MSC.

P8

Dual role of single residue in the synthetic site of isoleucyl-tRNA synthetase: ensuring specificity of synthetic site reactions and maintaining communication with the editing site

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Isoleucyl-tRNA synthetase (IleRS) covalently attaches isoleucine to cognate $tRNA^{Ile}$ in a two-step reaction. First, isoleucine is activated with ATP to form Ile-AMP. Then, isoleucine moiety is transferred to $tRNA^{Ile}$. Due to structural similarity, noncognate valine is often recognized as a substrate in both activation and transfer reaction. To ensure fidelity of translation and prevent mistranslation, IleRS evolved two proofreading mechanisms: hydrolysis of Val-AMP in the synthetic site (pretransfer editing) and hydrolysis of Val-$tRNA^{Ile}$ in the distant editing site (post transfer editing). Gly56 is a strictly conserved synthetic site residue that has already been shown to be important for amino acid discrimination in the activation reaction. Here we extend its importance to other reactions of IleRS. Gly56Ala variant efficiently hydrolyses Ile-AMP, revealing that, besides losing specificity in activation, it also lost specificity in the pre-transfer editing. Furthermore, Gly56Ala mutation affects the delivery of Val-$tRNA^{Ile}$ from the synthetic to the editing site. This implies that Gly56 influences distribution of the tRNA's 3' end among the synthetic and editing site and in this way modulates tRNA-dependent communication of two active site.

P9

The use of therapeutic suppressor tRNAs for Duchenne muscular dystrophy and dilated cardiomyopathy

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Nonsense mutations, in which coding triplets are converted to premature stop codons, represent the leading cause of genetic disease at the molecular level. In Duchenne Muscular Dystrophy (DMD), nonsense mutations are ~12% of all cases. In dilated cardiomyopathy caused by titin truncations (DCM-TTNtv), ~40% are nonsense mutations. These mutations are particularly devastating as they have dual effects on gene expression. First, as no corresponding tRNA exists for the three stop codons, ribosomes terminate translation prematurely, to produce truncated, nonfunctional polypeptides. Second, mRNAs with nonsense mutations have a shorter half-life than normal mRNAs due to nonsense-mediated decay. The impact of premature translation termination coupled with accelerated mRNA degradation makes disease-causing nonsense mutations exceptionally challenging to address therapeutically. Unlike lower organisms, mammals possess no naturally occurring suppressor tRNAs. This absence presents a therapeutic opportunity: introduced suppressor tRNAs could be used to treat genetic diseases caused by nonsense mutations without interfering with endogenous cellular processes. The ability to decode disease-causing stop codons and restore the normal amino acid would permit readthrough and facilitate a normal full-length protein. Here we show that introducing suppressor tRNAs can restore full-length protein and repair protein dysregulation in both in vitro and in vivo models of nonsense-mediated disease. Importantly, suppressor tRNAs did not induce native stop codon readthrough, reflecting the cell's intrinsic ability to distinguish between pathological nonsense mutations and physiological stop codons. Because suppressor tRNAs target a class of mutations rather than a specific gene or protein, this approach has broad therapeutic potential across diseases caused by nonsense mutations and this data establishes suppressor tRNAs as a promising strategy for nonsense mediated disease.

P10

Rapid growth and development of NARS1 patient directed research organization

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The Rory Belle Foundation, established in 2022, is the first nonprofit dedicated to patient-directed research for NARS1 disorder, a rare genetic condition identified in 2020. At the time of its founding, only 30 cases were documented worldwide, with minimal published research and a single active investigator. The Foundation initiated collaborations with RARE-X, a no-cost data collection platform, enabling caregiver-reported clinical and genetic data gathering. Early contributions from approximately 10 newly diagnosed families revealed previously unreported gastrointestinal issues in NARS1 patients, which were presented at the 2023 AARS Scientific Conference. Guided by a “Research Roadmap”, The Foundation’s engagement at scientific meetings catalyzed the expansion of its researcher network and supported investments in a biorepository, high-throughput drug screening, and targeted research grants. By leveraging principles of preclinical trial readiness—data collection, gap identification, and biomarker development—the organization has rapidly advanced the understanding and research capacity for NARS1 disorder. This model demonstrates the potential of patient-driven initiatives to accelerate progress in rare disease research.

P11

Exploring aminoacyl-tRNA synthetase-linked leukoencephalopathies: structural and biochemical insights into mitochondrial glutamyl-tRNA synthetase variants

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Mitochondrial aminoacyl-tRNA synthetases (mt-aaRS) are proteins responsible for adding an amino acid to the corresponding tRNA and, consequently, they are indispensable for the translation process in mitochondria. Interestingly, an increasing number of mt-aaRS protein variants are being identified, including in Portugal, linked to specific mitochondrial disorders (MD). One such disorder results from mutations in the EARS2 gene and is associated to Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL) (OMIM#612799), a rare genetic neurological disorder. Although the genetic cause of LTBL has been established, the molecular mechanisms underlying the disorder remain poorly understood. Hence, it is essential to clarify this by providing information on mt-aaRS structure, conformation, and function.

We aim to contribute to the field by studding the mitochondrial protein glutamyl-tRNA synthetase (EARS2), employing biochemical and biophysical methods to make for the first time, to our knowledge, a structural characterization of the human wild-type protein and three disease variants.

The proteins were heterologous expressed in E. coli and purified using chromatographic techniques, and later characterized in terms of structure and stability. Disease variants express at lower yields than wild-type protein. The EARS2-p.Glu96Lys variant was co-expressed with molecular chaperone GroEL/GroES enabling it's production. Purified EARS2-WT and EARS2-p.Glu96Lys present an identical folded conformation with the expected α-helical structure distinguished by far-UV CD. However, the variant present lower thermal stability, with an apparent melting temperature 4°C lower than wild-type.

We believe that establishing a heterologous expression and purification protocol for this human mt-aaRS will open new avenues for characterizing disease-associated variants and, in the future, contribute to the development of targeted therapies.

P12

Assessing the impact of chimeric tRNAs on tumorigenesis and tumor development using *Drosophila melanogaster* cancer models

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The aminoacylation of tRNAs is a key, tightly regulated process of the translation machinery. Most tRNA isodecoders need to be recognized by their aminoacyl-tRNA synthetase in both the anticodon and the acceptor stem in order to be aminoacylated. This is not the case for alanine (Ala), serine (Ser), tyrosine (Tyr), and leucine (Leu) isodecoders, where only the acceptor stem needs to be recognized. In these cases, mutations in the anticodon of the tRNA would lead to chimeric tRNAs, tRNAs that are charged with their corresponding amino acid, but that recognize a different anticodon, therefore introducing mutations throughout the whole proteome.

In a recent study in our lab, a comprehensive analysis of whole genome sequencing data from over 10,000 cancer and healthy tissue samples was conducted to study the extent of somatic mutations in tRNA genes. This work led to the discovery that tDNAs are hotspots of somatic mutations, which accumulate at up to nine-fold the rate observed in protein-coding genes. High levels of somatic mutations in tDNAs are found in the genome of tumor biopsy samples, including mutations resulting in chimeric tRNAs.

In this study we attempt to uncover the impact chimeric tRNAs have on tumorigenesis and tumor progression. In order to do so, a mistranslating Serine to Proline Drosophila melanogaster strain is crossed with strains mutant for different tumor suppressor genes, which result in the formation of tumors in the brain lobes of the fly. Through an allograft technique, the brain lobes of the larvae from this cross are injected in the abdomen of healthy host flies. This way, the tumorigenesis rate, tumor growth and lethality can be measured and compared. Furthermore, the tumoral tissue of an allograft can be recovered from the abdomen of the host fly, and reinjected into a new host. This process can be done indefinitely, thus allowing us to study the impact of chimeric tRNA in long-term standing tumors.

P13

Targeting highly secreted tryptophanyl-tRNA synthetase: a potential theranostic for hypercytokinemic severe sepsis

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In spite of extensive clinical and scientific endeavors, the mortality rate of sepsis remains high due to the lack of precise biomarkers for patient stratification and therapeutic guidance. Secreted human tryptophanyl-tRNA synthetase 1 (WARS1), an endogenous ligand for Toll-like receptors (TLR) 2 and 4 in response to infection, activates genes indicative of the hyperinflammatory sepsis phenotype. High plasma WARS1 levels stratified the early death of critically ill patients with sepsis, along with elevated levels of cytokines, chemokines, and lactate, as well as increased numbers of absolute neutrophils and monocytes, and higher Sequential Organ Failure Assessment (SOFA) scores. These symptoms were recapitulated in severely ill septic mice with hypercytokinemia. Moreover, the administration of WARS1 into moderately septic mice exacerbated morbidity and death. We developed an anti-human WARS1-neutralizing antibody (α-WARS1 mAb) that inhibits proinflammatory cytokine expression in marmosets with endotoxemia. The administration of this antibody to severely septic mice reduced cytokine storm, organ failure, and early death. With antibiotics, the antibody almost completely prevented fatalities. In minipigs with severe sepsis, α-WARS1 mAb reduced the worsening of SOFA scores and improved the P/F ratio while lowering the levels of proinflammatory cytokines and chemokines, as well as decreasing EPO production, AST/ALT levels, and the presence of inflammatory immune cells in the lungs. Collectively, the blood-circulating WARS1-directed anti-WARS1 treatment may provide an innovative theranostic approach for life-threatening hyperinflammatory sepsis.

P14

HARS deficiency is rescued by tRNA supplementation in a humanized yeast model of Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth (CMT) disease is the most common inherited peripheral neuropathy, resulting in severe motor and sensory nerve degeneration. Mutations V133F and Y330C in histidyl-tRNA synthetase (HARS) result in CMT type 2W (CMT2W); these mutations cause increased protein destabilization and impaired translation fidelity, ultimately resulting in distal sensory and motor dysfunction. We previously found that histidine supplementation unexpectedly exacerbated the negative phenotype of these two HARS mutants, despite alleviating phenotypic defects caused by HARS mutants V155G and S356N. Here, we use this well-established humanized yeast model, with high conservation of tRNA aminoacylation pathways, to determine whether increased availability of human cognate $tRNA^{His}$ would stabilize mutant HARS proteins and correct mistranslation. We show that both V133F and Y330C mutations induced protein instability and mistranslation at histidine codons, and that human tRNA*His* supplementation restored protein stability and fully rescued yeast growth phenotypes. This work further supports the therapeutic potential of cognate tRNA supplementation for CMT caused by HARS deficiencies, with further potential applications in treating other aminoacyl-tRNA synthetase related genetic disorders.

P15

Biogenesis of 5'-tRNA^{His} halves

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Angiogenin (ANG) is a stress-responsive ribonuclease that cleaves the anticodon loop of tRNA, generating tRNA halves. Among these, ANG-cleaved 5′-tRNA^{His} halves terminate with a UUG34 motif known to activate Toll-like receptor 7 (TLR7) and induce inflammation. tRNA^{His} is unique among tRNAs in acquiring a guanosine (G) at its 5′ end through post-transcriptional addition by THG1L. This modification is essential for its recognition and aminoacylation by histidyl-tRNA synthetase (HisRS). Interestingly, 5′-tRNAHis halves are selectively released from cells under stress conditions, with most of them lacking the 5' G+. The wobble position G34 of tRNA^{His} also undergoes queuosine (Q) modification by the QTRT1/QTRT2 complex, enhancing translation accuracy and efficiency, particularly under stress. Although the Q modification has been suggested to protect tRNAs from fragmentation, the mechanism and the impact of G addition and aminoacylation on 5'- tRNA^{His} biogenesis remain unclear. Recent studies show that ANG dissociates from its inhibitors under stress and binds to empty ribosomal A-sites. This interaction induces conformational changes that activate the RNase function of ANG, enabling efficient cleavage of both charged and uncharged tRNAs. Consistent with this mechanism, we were able to observe efficient production of 5' tRNA^{His} halves by ANG only in the presence of cell lysates. Using this method, combined with acid gel northern blotting, we evaluated the aminoacylation status of tRNA^{His} and the presence of the 5' G , with or without queuosine supplementation. Our results support a working model for the biogenesis of the 5'-tRNA^{His} halves. We propose that tRNA^{His} undergoes a sequential modification process, where the Q34 modification precedes 5'-G+ addition. Q34 hypomodification would lead to a non-productive association of HisRS with unmodified tRNA^{His}, cleavage by ANG at ribosomal A- sites, producing HisRS-bound nicked tRNA^{His} with G34 and without 5'-G+. Therefore, the apparent protective effect of Q modification on tRNA^{His} cleavage is through an indirect mechanism and requires the THG1L activity for the 5' G+ addition. Our findings may have implications for the pathogenesis of autoimmune antisynthetase syndrome (ASSD), in which HisRS is the most frequently targeted autoantigen.

P16

Exploring molecular adaptations of tRNA recognition in bacterial isoleucyl-tRNA synthetase type 2

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Accurate protein synthesis depends on the fidelity of aminoacyl-tRNA synthetases (aaRS), which charge tRNAs with their corresponding amino acids. Isoleucyl-tRNA synthetase (IleRS) is an enzyme that catalyzes the esterification of isoleucine to its cognate $tRNA^{Ile}$. Bacteria encode two types of IleRS, IleRS1 and IleRS2. These enzymes differ in the structure of their C-terminal domain, which consists of three subdomains (SD1-3) responsible for recognizing the anticodon of $tRNA^{Ile}$. Previous data (Brkic et al., Nat.Commun. (2023) 14:5498) revealed that IleRS2 contains an insertion within the SD2 and exhibits an evolutionary distinct SD3 fold compared to IleRS1. To explore how these structural differences impact $tRNA^{Ile}$ recognition, we determined the structure of IleRS2 from *Priestia megaterium* bound to $tRNA^{Ile}$ at 5.5 Å resolution and used that model for rigorous kinetic analysis. We demonstrated that SD3, despite its different folds, plays the same role in $tRNA^{Ile}$ recognition by both IleRS types. Furthermore, we unravel that SD2-insertion ($SD2_{ins}$) has a unique tRNA-binding role in IleRS2. Specifically, mutations in the $SD2_{ins}$ increased both K_M for $tRNA^{Ile}$ and k_{cat} by a similar extent, suggesting that the $SD2_{ins}$, acting as a remote-binding tRNA peptide, affects the rate-limiting Ile-$tRNA^{Ile}$ release step. Finally, we found that the $SD2_{ins}$ mutants, and even the enzyme lacking the entire $SD2_{ins}$, remain functional *in vivo* as they were able to rescue the growth of *E. coli* with inactivated IleRS. This raises intriguing questions about the origin and distribution of $SD2_{ins}$ in bacterial IleRSs and its potential role in adaptation to distinct modes of $tRNA^{Ile}$ recognition.

P17

Delivery of site-specific phosphorylated AKT1 to epithelial ovarian cancer cells prevents spheroid formation

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Epithelial ovarian cancer (EOC) is a major cause of gynecological cancer mortality, driven by aggressive metastasis and chemotherapy resistance. The PI3K/AKT pathway, often altered in EOC, regulates cell survival and proliferation, with AKT1 as a central node activated by phosphorylation at Thr308 and Ser473. To study its role in spheroid formation, a key step in metastasis, I use genetic code expansion to generate site-specific phosphorylated AKT1 variants. An orthogonal translation system (OTS), including phosphoseryl-tRNA synthetase, mutant $tRNA^{Sep}$, and an elongation factor EF-Sep, reassigns the UAG codon to incorporate phosphoserine (pSer) at position 473 with high specificity (Balasuriya et al., J Biol Chem. (2018) 293: 10744-10756). To produce $pAKT1^{S473}$, the OTS is co-expressed using a pCDF-duet1 vector that includes AKT1 TAG473 but does not contain the upstream kinase PDK1 gene. Meanwhile, expressing the duet vector containing AKT1 and PDK1 enables phosphorylation at T308 (Balasuriya et al., J Biol Chem. (2018) 293: 10744-10756). To produce doubly phosphorylated AKT1, the OTS is co-expressed with the duet vector that encodes both AKT1 TAG473 and the PDK1 gene. In addition, I fuse AKT1 with a TAT-tag to enable efficient protein delivery into EOC cells (Siddika et al., Cells (2022) 11). Delivery of TAT-$pAKT1^{T308}$ inhibits spheroid formation in OVCAR8 cells, while delivery of wildtype and serine phosphorylated AKT1 variants shows limited morphological effects. Notably, TAT-$pAKT1^{T308}$ treated cells maintain stable viability over 72 hours, while control and wildtype groups decline—likely due to the reactivation of endogenous AKT1 that promotes cell survival, confirmed by western blot. These findings reveal the critical role of AKT1 phosphorylation in EOC progression and suggest that site-specific modifications, such as at T308 vs S473, differentially regulate downstream signaling involved in spheroid formation, highlighting potential therapeutic targets to disrupt EOC metastasis.

P18

Peptide–RNA coacervates enhance protoribosome function: implications for the origin of translation

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The ribosome, due to its exceptional evolutionary conservation, preserves within its core a molecular fossil—the protoribosome—centered around the peptidyl transferase center (PTC), the ancient catalytic site responsible for peptide bond formation. While previous studies have demonstrated that RNA alone can exhibit PTC activity (Bose et al., NAR (2022) 50:1815–1828), the contribution of ribosomal protein fragments (rPeptides) to early ribosomal function remains largely unexplored. In this work, we investigate the role of rPeptides in primitive ribosomal systems and uncover their essential function in promoting the structural integrity and coacervation of two evolutionary models of the protoribosome. We show that rPeptides drive RNA coacervation across a wide concentration range, in a manner highly dependent on RNA sequence and structure (Codispoti et al., NAR (2024) 52:12689–12700). This peptide-induced compartmentalization not only stabilizes RNA but also protects it from degradation—creating a favorable microenvironment for sustained catalytic activity. Most importantly these conditions could enhance protoribosomal activity, supporting the notion that simple peptides may have played a pivotal role in the emergence of translation. Our research suggests a plausible scenario in which peptide–RNA interactions facilitated the formation of functional protocellular assemblies, marking a critical step in the transition from chemistry to biology. This work provides new insights into RNA-peptide cooperation during life's origins and the molecular evolution of the ribosome.

P19

An allelic series at R329 AARS1 reveals loss-of-function intolerance and variable dominant-negative effects

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Charcot-Marie-Tooth (CMT) disease is the most common inherited peripheral neuropathy, affecting approximately 1 in 2,500 individuals. Typically inherited in an autosomal dominant manner, CMT exhibits substantial phenotypic and genetic heterogeneity, with over 130 genes implicated. Among these genes, several aminoacyl-tRNA synthetases (ARSs)—enzymes that catalyze the charging of tRNAs with cognate amino acids—have been linked to dominant forms of CMT. Alanyl-tRNA synthetase (*AARS1*), which ligates alanine to tRNA in the cytoplasm, is one such ARS.

A recurrent pathogenic *AARS1* variant, R329H, has been identified in 11 families with dominant axonal CMT. Previous studies have shown that R329H causes both loss-of-function (LOF) and dominant-negative (DN) effects, although conflicting reports have raised questions about the extent of LOF associated with this variant. To clarify the functional role of R329 and better define the mechanism of disease, we generated an allelic series encompassing all possible amino-acid substitutions at position R329 and assessed each variant for both LOF and DN effects using an established humanized yeast complementation assay. All substitutions resulted in a complete loss of function, confirming that arginine at position 329 is essential for *AARS1* function. This supports the hypothesis that R329H-associated pathogenicity arises from impaired *AARS1* function.

When testing each LOF variant for DN effects, preliminary data suggest variant-dependent variability in DN effects. These findings suggest that while all R329 variants interfere with *AARS1* function, some LOF variants may not have dominant-negative effects due to decreased dimerization or decreased protein expression, pointing to a potential separation of function across the allelic series.

This work highlights the importance of R329 in *AARS1* function, demonstrating that all substitutions at this residue result in LOF and variable DN effects in our humanized yeast assay.

P20

Translation-mediated selective motor neuron degeneration in CMT- pathology associated with mutations in aaRS

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Dominant missense mutations in several aminoacyl-tRNA synthetases (aaRSs) are linked to axonal forms of Charcot-Marie-Tooth (CMT) disease, but the molecular mechanisms by which these otherwise ubiquitously expressed enzymes cause selective motor neuron degeneration remain elusive. To investigate this, we use human small molecule neural precursor cells (smNPCs) differentiated into motor neurons and integrate multiple approaches, including (i) subcellular RNA-seq and Ribo-seq profiling and a microscopy-based assay to assess global and compartment-specific effects (between soma and neurites) on translation; (ii) single-molecule displacement mapping to assess diffusion differences in soma and neurites that impact aminoacyl-tRNA turnover between aaRS and ribosomes; (iii) biochemical analysis to dissect kinetic properties of CMT-aaRSs; and (iv) AI-based approaches to assess the integrity of the tRNA binding surface of CMT-aaRSs. Together, our findings point to a converging mechanism in which CMT mutations impair translation through tRNA sequestration, that is amplified in neurites due to constrains in abundance of translational components. Our integrative approach provides new insight into the cellular specificity of aaRS-associated neurodegeneration and selective motor neuron degeneration and establishes the framework for therapeutic intervention with selective administration of cognate tRNAs.

P21

NSUN7 is an RNA:m^{5}C catalytically inactivate protein that regulates spermatozoa flagellum assembly

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RNA is an important macromolecule in living organisms and serves as a critical role in the maintenance of life activities. To date, more than 170 types of chemical modifications have been found, which enhance the complexity of RNA functions and broaden the scope of "RNA epitranscriptomics". 5-methylcytosine (m^{5}C) is among the most abundant RNA modifications, drawing significant attention for its extensive distribution across diverse RNA species and its implication in various human diseases. The intracellular RNA:m^{5}C modification is mainly catalyzed by NSUN protein family. The NSUN family comprises seven members, named NSUN1-7. Among NSUN family, all members except NSUN7 have had their RNA substrates and biological functions been extensively studied. To date, the specific catalytic activity and RNA substrates of NSUN7 remain controversial. Defects of *Nsun7* lead to male infertility in human and mouse, and the pathogenic mechanism has not yet been elucidated. In this study, we found that NSUN7 is predominantly enriched in the adult mouse testis, particularly in the elongated spermatid. Through transcriptome- wide profiling of RNA:m^{5}C modifications using UPLC-MS/MS and bisulfite RNA sequencing, we unexpectedly found that knockout of *Nsun7* does not affect m^{5}C modification sites or levels on mRNA, rRNA, tRNA, or other RNA species. In *Nsun7* knockout mice, the progressive motility of mature spermatozoa was seriously impaired, accompanied with the defects in spermatozoa microtubule structures and mispositioning of longitudinal columns. Additionally, we have found that knockout of *Nsun7* results in a significantly decrease of a cohort of mRNAs which related to cilium composition. Our findings suggested that NSUN7 is the only member in NSUN family which does not possess RNA:m^{5}C catalytic activity, and could regulates spermatozoa flagellum assembly in a manner independent of RNA:m^{5}C modification.

P22

Deciphering the relationship between two arginyl-tRNA synthetases from the predatory bacteria *Herpetosiphon aurantiacus*

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Arginyl-tRNA synthetase (ArgRS) is a member of the predominantly monomeric class I family of aminoacyl-tRNA synthetases, which display a signature HIGH motif in the active site. It is known that alterations of this key catalytic motif can unlock new features (Brkic *et al*., *Nat. Commun.* (2023) 14, 1-12). We found that the predatory bacteria *Herpetosiphon aurantiacus* has two genes for arginyl-tRNA synthetase: a typical HaArgRS1 with an HVGH motif, and an atypical HaArgRS2, with an RVDH motif instead. HaArgRS1 actively performs aminoacylation, while HaArgRS2 apparently does not bind ATP and consequently cannot perform the canonical function. To explore alternative HaArgRS2's roles, we searched for its putative protein interactors utilizing a pull-down assay followed by mass spectrometry. The analysis revealed that HaArgRS1 is the most probable interactor of HaArgRS2. The HaArgRS1:HaArgRS2 interaction was further confirmed by microscale thermophoresis and characterized as a seemingly transient due to a low binding affinity. The latter is in line with the current knowledge that ArgRSs typically do not form functional dimers and suggests that HaArgRS2 may have a regulatory function over HaArgRS1. Surprisingly, we observed that HaArgRS2 also binds *Escherichia coli* ArgRS, with an order of magnitude higher affinity than it has for HaArgRS1. Thus, HaArgRS2 may be involved in a predatory strategy of *H. aurantiacus*. Further research is needed to confirm the nature of this relationship and shine light on its tuning.

P23

Investigating the underlying molecular basis of CMT-causing AlaRS pathogenic alleles by using *S. cerevisiae* as a model organism

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Charcot-Marie-Tooth (CMT) disease, a hereditary sensory and motor neuropathy affecting 1 in 2500 people, has no disease-specific treatment available at present. Patients present with classical CMT phenotypes characterized by distal muscle weakness and atrophy, nerve pain, balance problems, and sensory loss (M. Mahmood et al., IUBMB Life. (2025) 77:e70017). One family of genes implicated in CMT are the enzymes known as the aminoacyl-tRNA synthetases (aaRSs). aaRSs are highly conserved and essential enzymes that ligate the amino acids to their cognate tRNAs during protein synthesis, thus ensuring translational fidelity. Pathogenic CMT mutations in aaRSs are inherited in an autosomal dominant manner and result in progressive chronic degeneration of peripheral axons. It can lead to enzyme destabilization, reduced aminoacylation efficiency, or mis-aminoacylation of the cognate tRNAs (R. Meyer-Schuman et al., Hum Mol Genet. (2017) 26:R114-R117). To study CMT phenotypes resulting from impaired translation, we created a humanized yeast (S. cerevisiae) CMT model for alanyl-tRNA synthetase (AlaRS) by replacing the yeast endogenous Ala1 gene with the human wildtype AlaRS1. Results showed that human wildtype AlaRS1 supported yeast growth at 30 and thus complemented the deletion of Ala1. Furthermore, two CMT-associated AlaRS alleles; R329H and G102R also supported yeast growth, albeit significantly slower than cells expressing AlaRS wildtype. Additionally, both alleles showed reduced functionality in yeast cells when co-expressed with the wildtype, indicative of the presence of a dominant-negative phenotype. The findings support previous evidence regarding the pathogenicity of these variants, however our model showed that the presence of these alleles does not result in total loss-of-function in yeast. Therefore, our system will allow us to investigate the cellular and molecular basis of the pathogenic AlaRS variants and develop targeted therapeutic approaches for CMT patients.

P24

Kinetic mechanism of *E. coli* IleRS determined by tRNA modifications

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IleRS catalyzes aminoacylation of Ile to both Ile(CAU) and Ile(GAU) in *E. coli*. While crystal structures of IleRS in complex with tRNAIle demonstrate that it is a class I enzyme, the kinetic mechanism of the enzyme has remained unknown for decades. Additionally, while IleRS requires the lysidine modification at position 34 (L34) to discriminate against mis-aminoacylation of tRNA Met(CAU), the importance of the network of tRNA modifications is unknown. Here we show that the kinetics of aminoacylation of *E. coli* IleRS is dependent on a network of tRNA modifications. It not only requires L34 to perform efficient aminoacylation of tRNA Ile(CAU), but also it requires the t6A37 modification at position 37. Importantly, we show that, consistent with other class I enzymes, IleRS exhibits burst kinetics on both Ile(CAU) and Ile(GAU), indicating product inhibition. Interestingly, we observe this product inhibition only with the further modified ct6A37, which is the native modification in both Ile(CAU) and Ile(GAU). The results demonstrate the importance of ct6A37 as a determinant for a higher affinity of IleRS to the charged Ile-tRNA^{Ile} product in order to facilitate product delivery to the ribosome protein synthesis machinery.

P25

Selection of pyrrolysin-tRNA synthetase mutants for the cotranslational incorporation of boronic acid side chains into an Anticalin-type sugar-binding protein

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Protein-carbohydrate interactions are essential in many biological processes, but are often weak due to the strongly hydrophilic and flexible nature of oligosaccharides. Boronic acids, which have long been known to form covalent diesters with vicinal diols, can enhance carbohydrate binding. To exploit this, the non-canonical amino acid *p*-borono-L-phenylalanine (Bpa) was site-specifically incorporated into the ligand pocket of human lipocalin 2, thus generating a "Borocalin" (Edwardraja et al., ACS Synth. Biol. (2017) 6:2241). This was achieved by amber suppression in *E. coli* using an engineered Bpa-specific aminoacyl-tRNA synthetase (aaRS) (Schiefner et al., Biochem. (2018) 57:2597) in combination with an orthogonal suppressor tRNA. Further engineering of the lipocalin scaffold led to improved boronic acid accessibility, enabling binding of monosaccharides and of an aromatic diol compound with micromolar affinities. pH dependence of the complex formation and X-ray structural analysis indicated that the anionic tetrahedral form of Bpa is more reactive towards 1,2-*cis*-diols (Sommer et al., ChemBioChem (2020) 21:469).

Therefore, we set out to replace Bpa by L-benzoxaborol-alanine (Bxa), which should favour the formation of cyclic diesters with sugars at physiological pH. While Bxa turned out not to be recognized by the Bpa-specific aaRS, we found that a mutated *M. mazei* pyrrolysyl-tRNA synthetase, previously engineered for *p*-(phenylazo)-L-phenylalanine (Pap) as substrate (Mayrhofer et al., Nat. Commun. 2024 15:10693), showed detectable incorporation of Bxa. This enzyme has been subjected to random mutagenesis and fluorescence-activated cell sorting (FACS), utilizing sfGFP carrying an amber stop codon as reporter protein, in order to select optimized PylRS variants for Bxa incorporation. The resulting aaRS will be tested for Bxa incorporation into the Borocalin, followed by reshaping the ligand pocket to achieve specificity towards biologically relevant oligosaccharides.

P26

Architectural basis of tRNA-dependent sterol aminoacylation

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Ergosteryl-3β-O-L-aspartate synthase (ErdS) catalyzes tRNA-dependent aspartylation of ergosterol, a lipid essential for fungal cell membrane integrity. Yet, the molecular basis of this reaction has remained elusive. Here, we determined the cryo-electron microscopy structure of ErdS and uncovered an unprecedented sterol- binding pocket and a tRNA-guided intramolecular aminoacyl transfer mechanism. The CCA end of $tRNA^{Asp}$ undergoes a large displacement to reach the aa-tRNA transfer active site, while the tRNA elbow is stabilized by a long extension of the N-terminal α-helix. Structural and mutational analyses demonstrate that domain fusion, dynamic repositioning, and tRNA-mediated substrate handover collectively enable the multifunctional catalytic efficiency of ErdS. These findings elucidate a unique mechanism of tRNA-dependent sterol modification and provide novel insights into fungal membrane dynamics, highlighting potential targets for antifungal therapies.

P27

Analysis of Charcot-Marie-Tooth-associated tyrosyl-tRNA synthetase variants of unknown pathogenicity in *Saccharomyces cerevisiae*

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Charcot-Marie-Tooth disease (CMT) is a genetically diverse hereditary disorder that affects the motor and sensory nerves. Aminoacyl-tRNA synthetases (aaRSs) play an important role in the genetic basis of CMT2, with more than 60 disease-causing alleles identified across eight different aaRSs, including alanyl-, asparaginyl-, glycyl-, histidyl-, methionyl-, seryl-, tryptophanyl-, and tyrosyl-tRNA synthetases. *Saccharomyces cerevisiae* has become an important model system for investigating the molecular and cellular mechanisms underlying pathogenic aaRS mutations associated with CMT. This simple system enables systematic screening of variants deposited in clinical genomic databases, such as the National Institutes of Health (NIH) ClinVar database, to evaluate the potential pathogenicity and underlying mechanisms of aaRS mutations. In tyrosyl-tRNA synthetase (*YARS1*), five dominant variants, G41R, D81I, ΔVKQV 153–156, E196K, and E196Q have been linked to CMT2. However, many additional *YARS1* variants listed in the NIH ClinVar database are associated with CMT patients without confirmed pathogenicity. Using the ClinVar database, we screened for *YARS1* variants associated with CMT that have not been established as pathogenic. With the aid of computational prediction of mutation pathogenicity, we selected variants for further study in *S. cerevisiae*. Inserting these mutations into the *S. cerevisiae TYS1* gene and performing subsequent phenotypic analyses revealed potential disease-causing variants. Our results support the utility of large-scale screening of aaRS variants in yeast to provide further evidence of pathogenicity and to elucidate the cellular basis of disease.

P28

Inhibition of FARS with RNA from C9orf72 repeat expansion mutation associates with membrane protein alterations in ALS and FTD

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Our study explores the genetic basis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), with a particular emphasis on the widely occurring C9orf72 gene mutation. This mutation involves the expansion of a hexanucleotide repeat sequence - GGGGCC - transcribed in both sense (G4C2)n and antisense (C4G2)n directions, leading to the formation of nuclear RNA foci. Significant finding in our latest research is the discovery of an interaction between antisense C4G2 repeat RNA and phenylalanine-tRNA synthetase (FARS), resulting in reduced charging of phenylalanine-tRNA and consequent impact on the expression of proteins rich in phenylalanine content. Through bioinformatic analysis, we observed that the majority of proteins high in phenylalanine content localize to membranes, with the endoplasmic reticulum being notably prevalent. Utilizing techniques such as immunocytochemistry analysis and subcellular fractionation combined with western blots, we observed notable downregulation of these proteins in cells derived from C9orf72 patients. Our study highlights the significance of aminoacyl-tRNA synthetases and phenylalanine-rich proteins in C9orf72 ALS/FTD, suggesting their potential role in disease development. These innovative methods provide valuable tools for probing C9orf72 mutation-derived RNAs, paving the way for novel research avenues and therapeutic strategies.

Črnigoj et al., Nat. Commun. (2023) 14(1):5764

P29

Treatment of KARS1 deficiency with lysine supplementation in a pediatric patient: a three-year clinical update

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The field of medical genetics is rapidly changing from a primarily diagnostic specialty to having many therapeutic options, even for disorders that fall outside of the classical inborn errors of metabolism. One such group of disorders is the aminoacyl-tRNA synthetase (ARS1) deficiencies. Over the past several years, supplementation of the cognate amino acid has been trialed in various ARS1 deficiencies, including for LARS1, IARS1, MARS1, FARSA, FARSB, and SARS1.

Our group previously described our preliminary experiences with lysine supplementation in two patients with KARS1 deficiency. We now have almost three years of follow-up data on one of these patients, a now 8-year-old male with autism spectrum disorder, global developmental delay with history of developmental regression, myoclonic epilepsy, and mild chronic anemia. After starting lysine supplementation, he was initially able to wean down from three anti-seizure medications to one, but did eventually require restarting a second medication after recurrence of seizures. He continues to make developmental progress and has not had any further episodes of regression. His family reports continued subjective improvements in his behavior. To our knowledge, this is the longest-term follow up of treatment of KARS1 deficiency with lysine supplementation. While larger treatment cohorts are necessary to better understand its efficacy, lysine supplementation continues to seem like a well- tolerated, relatively low-risk intervention with potential benefits in KARS1 deficiency.

P30

P. falciparum threonyl-tRNA synthetase in drug discovery: strategies for construct design and stabilization

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Plasmodium falciparum threonyl-tRNA synthetase (*Pf*ThrRS) is a promising target for the development of antimalarial drugs (Lukarska and Palencia, Enzymes (2020) 48:321–350, Nyamai and Tastan Bishop, Malar. J. (2019) 18:1–27). However, the lack of an experimentally determined 3D structure makes rational inhibitor design particularly challenging. Moreover, the enzyme's high molecular weight, aggregation tendency, low catalytic activity, and a long disordered loop region present significant obstacles for structural studies (Victor et al., Malar. J. (2010) 9:1–13).

To address these challenges, we designed two modified *Pf*ThrRS constructs incorporating additional cysteine residues to stabilize the flexible loop region. Additionally, four fusion constructs were created to enhance solubility and improve folding. In parallel, two constructs were engineered for expression in insect cells using the baculovirus expression system, offering a eukaryotic-like environment for protein production.

Here, we present our current progress and preliminary results, highlighting construct design strategies and expression approaches aimed at enabling structural and functional characterization of *Pf*ThrRS for antimalarial drug discovery.

P31

Bacterial seryl-tRNA synthetases as therapeutic targets: a fragment- based approach to inhibition

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The rise of antimicrobial resistance highlights an urgent need for new antibacterial agents and innovative therapeutic targets. Microbial aminoacyl-tRNA synthetases (aaRS) have emerged as attractive option due to their essential role in protein synthesis. Mupirocin, an IleRS inhibitor and anti-infective agent, stands as a successful example already in clinical use (Kwon et al., Nat. Rev. Drug Discovery (2019) 18:629–650).

Seryl-tRNA synthetases (SerRS), validated as antibacterial targets over two decades ago through the natural product albomycin, have received comparatively less attention in drug development (Stefanska et al., J. Antibiot. (2000) 53(12):1346-1353). Most of the known SerRS inhibitors are structural mimics of their natural intermediate, seryl-AMP (Cain et al., J. Med. Chem. (2019) 62(21):9703-9717). Although these analogues often exhibit activity even in the nanomolar range, they lack selectivity between bacterial and human enzymes, limiting their clinical potential. To address this, we utilized fragment-based screening to identify novel, chemically diverse inhibitors with improved selectivity.

Our work focused on *Escherichia coli* SerRS due to its clinical relevance as a multidrug-resistant pathogen. *Ec*SerRS, as well as human and *Staphylococcus aureus* SerRS, was prepared via recombinant overexpression in *E. coli*, then purified using affinity and size exclusion chromatographies. We used thermal shift assay to screen *Maybridge* Ro3 fragment library followed by hit validation with ^{1}H NMR spectroscopy, then proceeded to screen *Enamine* Fluorinated fragment library with ^{19}F NMR. Both screenings yielded more than 30 hits in total. We then studied binding positions of the active fragments using both computational and crystallographic data to design more potent inhibitors based on the fragment structures. Our study lays the groundwork for future optimization into lead compounds and highlights the potential of SerRS in antibiotic development.

P32

Characterizing pathogenic mutations in asparaginyl-tRNA synthetase

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Pathogenic mutations in asparaginyl-tRNA synthetase cause a pediatric disorder with varying symptoms, which is commonly summarized as NARS disease. To better understand the molecular and cellular mechanism by which disease-associated NARS variants cause these disorders, we investigated the properties of WT and disease- associated NARS variants using both protein biochemistry and human cell models. Our data show that thermal instability is likely not the mechanism by which specific disease-associated NARS variants exhibit their pathogenicity, but disease-causing mutations may alter NARS activity. Our work in human cell lines suggests that overexpression of disease-associated NARS variants is cytotoxic and causes an activation of the integrated stress response (ISR) as reflected by elevated p-eIF2a levels. We tested several therapeutic approaches to ameliorate cytotoxicity and found that mutations respond to therapeutic intervention in an allele-specific manner. These findings provide insight into the mode of action for specific NARS variants and raise the potential for a clinical intervention.

P33

Analyzing human aminoacyl-tRNA synthetase mutants using yeast

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Aminoacyl-tRNA synthetases are housekeeping enzymes that produce aminacylated tRNAs for protein synthesis. They are also considered to be the guardians of the genetic code but throughout decades, they have been associated with a myriad of functions outside translation. These range from various aspects of cellular homeostasis (DNA repair, splicing, transcriptional and translational regulation,etc…) to retroviral replication. It is therefore not surprising that aminoacyl-tRNA synthetases have been found to be related to various diseases in humans and/or involved in potential crucial aspects of human pathogens. Nevertheless, it is of importance to evaluate the synthetase activity using a simple growth test. To this aim, we are currently creating a whole yeast collection of strains in which the human synthetase is expressed under control of the corresponding yeast promoter/terminator. We have encouraging results for some aminoacylation systems, but for the majority there seems to be a requirement to co-express the corresponding human tRNA. We combine the growth analysis with a structural approach using either 3D models or real enzyme structures. During the last years it has become obvious that so-called cytosolic aminoacyl-tRNA synthetases are multi-localized enzymes and this is of importance when analyzing mutants that are involved in cellular dysfunctions. To get insights of the enzyme's localization, we take advantage of our split-GFP localization systems which offer the opportunity to visualize only a fraction a the aminoacyl-tRNA synthetase pool (outside the one that is cytosolic).

P34

Non-canonical functions of mitochondria-relocated cytosolic aminoacyl-tRNA synthetases in health and disease

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It is widely established that cytosolic aminoacyl-tRNA synthetases ($_{cyto}$aaRS), besides their role in tRNA aminoacylation, exert diverse nontranslational functions which are usually associated with relocation to other subcellular compartments (Yakobov et al., Biochim. Biophys. Acta (2017) 186: 387-400). These roles are crucial for cell homeostasis as $_{cyto}$aaRS genetic variants are associated with a myriad of diseases (Turvey et al., Front. Physiol. (2022) 13:1029218). Unfortunately, a large cohort of patients lacks effective treatments, since the underlying affected molecular mechanisms are not understood. Considering that these diseases frequently affect the nervous system, we wondered to what extent some of them could be explained by a $_{cyto}$aaRS variant-induced mitochondrial dysfunction. To gain insight we evaluated, as a first approach, the mitochondrial localisation of different wild-type $_{cyto}$aaRS in HEK293T cells. Immunodetection on highly pure mitochondrial extracts showed an association between cytosolic methionyl-tRNA synthetase (MARS1) and the mitochondrion outer membrane. Therefore, we aim to characterise MARS1 mitochondrial localisation, decipher in which molecular mechanisms it may intervene and the role it could play in the pathogenicity observed in patients bearing variants of this enzyme.

P35

The use of genetic code expansion in the production of highly stable protein nanopores

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With more than 200 different non-canonical amino acids (ncAAs) that can be introduced into proteins by stop codon reassignment, genetic code expansion offers a unique approach to creating proteins with altered properties. With this approach, halogenated ncAAs can be used to stabilize proteins that have to withstand harsh conditions, which can be necessary in certain processes and techniques. In particular, for protein analysis using nanopore technology, it would be advantageous to create highly stable biological nanopores that allow us to unfold the protein analyte while the nanopore itself remains intact.

To this end, we have produced stabilized oligomeric pores of the pore-forming protein bryoporin (Bry) containing one of two ncAAs at different positions. Several Bry variants were prepared containing either *m*-Cl-tyrosine (ClY) or *p*-pentafluorosulfanyl-phenylalanine (SF5). We tested their ability to oligomerize with native PAGE and checked their stability in guanidinium chloride (GdmCl) using nano-differential scanning fluorimetry (nanoDSF). Isolated pores of the most stable variants were then prepared and characterized by cryo-EM and single channel recordings on planar lipid bilayers.

While nanopores of the SF5-containing Bry variants proved to be more stable in nanoDSF experiments, we could not use them for further analysis due to their poor solubility and high aggregation tendency. The nanopores of the ClY-containing variants, on the other hand, were easy to produce but generally exhibited lower stability. With the most stable ClY-containing variant, we were able to perform single-channel recordings in buffers containing up to 3 M GdmCl, whereas with the wild-type Bry, which does not contain ncAAs, recordings were only possible in buffers containing up to 1.5 M GdmCl. These results provide a positive outlook for our future prospects of achieving translocation and identification of full-length native protein analytes.

P36

Human tRNA anticodon variants exceed read-through threshold for phenotypic rescue of an ALS-causative premature termination codon

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Nonsense mutations make up over 20% of single nucleotide substitutions that cause disease, including frontotemporal dementia, Charcot Marie Tooth disease, and amyotrophic lateral sclerosis (ALS). Many of these diseases have no cure or effective treatment. The arginine CGA to TGA mutation is the most frequent disease- causative premature termination codon (PTC), making up 21% of pathogenic PTCs. The same nonsense mutation at codon 495 in the fused in sarcoma (FUS) gene has been shown to cause ALS. While wild-type FUS participates in nuclear mRNA processing, the arg-to-TGA mutation truncates FUS and eliminates its nuclear localization signal, causing cytoplasmic aggregation and selective motor neuron death. Using arginine transfer RNA ($tRNA^{Arg}$) anticodon variants, we restored full- length wild-type FUS sequence from the ALS-causative FUS-R495X allele in cellular models of disease. Due to the lack of key aminoacylation identity elements in $tRNA^{Arg}$ anticodon, nonsense suppressor $tRNA^{Arg}UCA$ genes should selectively decode UGA stop codons, faithfully incorporate arginine, and eliminate their disease phenotypes. A panel of nonsense suppressor $tRNA^{Arg}$ derived from natural human $tRNA^{Arg}UCG$ genes were developed, demonstrating differential levels of readthrough in a neuroblastoma model of ALS. All suppressors tested led to full-length FUS rescue, confirmed by immunoblot and mass spectrometry; however, restoration of nuclear localization and reduction of aggregation was dependent on the $tRNA^{Arg}$ gene used for FUS-R495X suppression, with 13% rescue of FUS needed to reverse FUS aggregation and mislocalization. Overall, tRNAs offer a unique opportunity to restore wild-type FUS, reduce its associated toxic gain of function, as well as rescue its canonical function. Additionally, suppressor $tRNA^{Arg}$ allow for a platform to determine a therapeutic threshold of full-length wildtype FUS needed for effective treatment, further enriching our understanding of ALS disease pathology.

P37

Development of an aminoacyl-tRNA synthetase for the incorporation of 7-hydroxycoumaryl-alanine

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Fluorescent non-canonical amino acids have gained wide interest for studying proteins and biological processes. The co- translational incorporation of such artificial side chains enables targeted protein labeling with fluorescent probes without disrupting their natural structure or function. Owing to its excellent photophysical properties, including a strong fluorescence signal combined with high environmental sensitivity, the compound L-(7-hydroxycoumarin-4-yl)-ethylglycine (7-HCAA) has been introduced as a marker in protein analysis and fluorescence spectroscopy (Wang et al., J. Am. Chem. Soc. (2006) 128: 8738–8739).

However, 7-HCAA's extended side chain, including an additional Cγ-Cδ single bond, leads to an unnaturally high degree of conformational freedom and potential sterical hindrance, which may interfere with protein folding and/or ligand binding. 7-hydroxycoumaryl-alanine (HCA), on the other hand, offers a structurally optimized side chain, similar in size to natural aromatic amino acids, with the same photophysical properties.

The aim of this project is to select an aminoacyl-tRNA synthetase for the incorporation of HCA using directed evolution. To this end, error-prone PCR is used to generate genetic variations in the active site of the pyrrolysyl-tRNA synthetase (PylRS). Flow cytometry is then performed to select mutants towards the new substrate specificity (Mayrhofer et al., Nat Commun (2024) 15:10693).

Preliminary results suggest that the concomitant incorporation of HCA at genomic amber stop codon positions in *E. coli* may trigger a toxic effect, possibly induced by the laser light during cell sorting. A modified workflow, where PCR is performed on the bacterial cells after each sorting cycle, prevents the loss of clones that harbour an active PylRS mutant. Successful HCA incorporation into binding proteins should yield novel diagnostic tools, for instance, by integrating HCA into an antibody fragment or anticalin near the ligand-binding site.

P38

tRNA-derived SINE repetitive elements regulate porcine embryonic genome activation

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Porcine embryonic genome activation (EGA) occurs at the 8-cell stage, in contrast to mice, where it occurs at the 2-cell stage. To investigate the differences in the timing of EGA between pigs and mice, we optimized 4sU incorporation of nascent transcripts with single-cell TimeLapse-seq to precisely define the EGA genes and repeat elements in pigs and mice. We found that porcine EGA genes share low similarity with murine EGA genes but are more similar to genes activated during the 4-8 cell stages in mice, many of which are related to ribosome biogenesis. Further studies revealed that the activation of porcine EGA genes may be coupled with the activation of SINE transposons, whereas murine EGA is associated with LTR activation, and SINEs in mice are activated in the post-EGA stage. By analyzing the distance between genes and transposable elements (TEs), we observed that SINEs within 5kb of porcine EGA and post-EGA genes, as well as murine post-EGA genes, show higher correlation with gene transcription than other TEs, and these SINEs are more likely to be transcribed independently. Genes co-transcribed with SINEs in both pigs and mice are enriched in ribosomal genes, despite that SINEs in pigs and mice are highly divergent evolutionarily. Additionally, porcine, but not murine EGA genes are surrounded by more actively transcribed SINE elements, the vast majority of which belong to the PRE1 family derived from tRNA. Suppression of RNA polymerase III (Pol III) can inhibit the transcription of these SINEs, and we also found that the transcription of EGA genes is suppressed, leading to reduced embryonic developmental rates. These results indicate that SINEs are critical elements regulating porcine, but not murine, EGA gene activation.

P39

Inducing bacteria to self-destruct by hijacking the tRNA-charging process

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Aminoacyl-tRNA synthetases (aaRSs) play an essential role in protein synthesis by catalysing the covalent attachment of amino acids to their cognate tRNAs. Bacteria are highly reliant on protein synthesis as they undergo rapid growth and division, making aaRSs attractive targets for antibacterial drug development. Mupirocin, an isoleucyl-tRNA synthetase inhibitor, is a common topical antibiotic used to treat skin infections.

We previously discovered that tyrosyl-RS (TyrRS), a class I aaRS, is the target of a series of nucleoside sulfamate inhibitors in malaria parasites. TyrRS catalyses the formation of a highly inhibitory tyrosine-inhibitor conjugate at the active site via a novel mechanism we named reaction hijacking. Furthermore, we identified OSM-S-106, a compound with a chemical scaffold distinct from nucleoside sulfamates. This compound inhibits parasite asparaginyl-RS (AsnRS), a class II aaRS, via the enzyme-mediated production of an Asn-OSM-S-106 adduct. Hence, reaction-hijacking inhibitors have broadly applicable activity against both classes of aaRSs. Here we assessed the minimum inhibitory concentration (MIC) of reaction-hijacking inhibitors against a panel of clinically relevant Gram-positive and Gram-negative bacterial strains. Notably, *Escherichia coli* and *Enterococcus faecium* exhibited high susceptibility to the inhibitors. Targeted mass spectrometry of cellular extracts revealed four aaRS targets in both *E. coli* and *E. faecium*: prolyl-RS, tyrosyl-RS, glutamyl-RS, and threonyl-RS. We demonstrated that OSM-S-106 potently inhibits recombinant bacterial prolyl-RSs. Enzyme kinetics assays and thermal stabilisation assays confirmed reaction hijacking as the mechanism of inhibition. Moreover, OSM-S-106 inhibits bacterial growth with low toxicity to human cell. Our findings highlight a promising new avenue for the development of anti-infectives.

P40

Development of cyclic peptide inhibitors targeting system Xc via aminoacyl-tRNA synthetase–mediated synthesis and structural characterization by cryo-EM

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System Xc is a heterodimeric amino acid transporter composed of xCT (SLC7A11) and CD98hc (SLC3A2) that mediates the exchange of extracellular L-cystine for intracellular L-glutamate. This transporter is highly expressed in various cancer cells and cancer stem cells (CSCs), where imported L-cystine is reduced to L-cysteine, promoting glutathione synthesis and contributing to cellular antioxidant defense. This process plays a critical role in the survival and therapeutic resistance of cancer cells.

In this study, we aimed to develop cyclic peptide inhibitors targeting System Xc using the RaPID (Random nonstandard Peptides Integrated Discovery) system. The RaPID platform enables the in vitro synthesis and screening of diverse libraries of cyclic peptides derived from natural amino acids by artificially controlling the specificity of aminoacyl-tRNA synthetases (aaRSs). This technology allowed efficient identification of peptides with high affinity for the System Xc complex.

Through screening and surface plasmon resonance (SPR) binding assays, nine cyclic peptides exhibiting strong binding to the xCT–CD98hc complex were identified. Furthermore, we determined the cryo-electron microscopy (cryo-EM) structure of the xCT–CD98hc complex bound to one of the cyclic peptides at 3.2 Å resolution. The structure revealed detailed molecular interactions at the peptide-binding site on xCT, providing insights into the mechanism of inhibition.

These findings demonstrate that combining aaRS-mediated peptide synthesis with high-resolution structural analysis offers a powerful approach to selectively target metabolic vulnerabilities in cancer stem cells. Selective inhibition of System Xc represents a promising therapeutic strategy for preventing tumor progression and recurrence.

P41

Expanding the genetic and clinical spectrum of neurological disorders associated with tRNA synthetase deficiencies in Russian patients

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Background and Aims: Aminoacyl-tRNA synthetases (ARSs) are a group of enzymes that catalyze the aminoacylation reaction of a specific amino acid and its cognate tRNA. Two decades ago, deficiency of an ARS was first associated with disease, clinically classified as a peripheral neuropathy. Human disease related to dysfunction of ARSs is increasingly recognized. Here we describe the results of two distinct investigations: in the first one we provided routine diagnostics of central/peripheral nervous system diseases among Russian patients; the second study focused specifically on *DARS2*-associated leukoencephalopathy. Some pathogenic variants in the genes encoding Aminoacyl-tRNA Synthetases (cytosolic and mitochondrial) we identified for the first time.

Methods: Genetic analysis was performed using massively parallel sequencing (target panels, whole exome or genome) and direct Sanger sequencing. Variant pathogenicity was evaluated following ACMG criteria using the Franklin Genoox platform.

Results: 14 patients with heterogeneous clinical presentations were diagnosed with mutations in 10 different genes encoding Aminoacyl-tRNA Synthetases (3 cases with *LARS2*, 2 cases with *IARS2* and *NARS2* respectively, and single cases with *EPRS1*, *FARS2, GARS1, KARS1, SARS2, TARS2, WARS2*). Nine variants were not previously described as pathogenic. With *DARS2*-associated leukoencephalopathy we identified 206 patients, revealing three distinct clinical forms based on age of onset (early childhood 49%, childhood 35%, late-onset 16%) and 13 novel *DARS2* variants, including a recurrent large deletion (exons 16-17) potentially specific to the Russian population.

Conclusions: Our study expands the spectrum of pathogenic variants in ARS-related disorders. These results support the role of comprehensive genetic screening and population-specific mutation patterns for effective diagnosis tRNA synthetases-associated diseases.

P42

Deciphering tRNA repertoires and translation coordination during mouse early embryogenesis by ORACLE-tRNAseq

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Beyond extensive epigenetic reprogramming following fertilization, translational control also plays a pivotal role during maternal-to-zygotic transition (MZT) in early development, which involves intricate coordination between codon demand and tRNA anticodon pool supply. However, due to current technical limitations, elucidating the landscape of embryonic tRNA pools – a critical nexus connecting the mRNA and protein – remains an underexplored challenge. Here, we developed Optimized Reaction for Accurate Capture of Low-input Entities tRNA Sequencing (ORACLE-tRNAseq), a novel methodology enabling robust tRNA profiling even from limited sample amounts, down to as few as 5 mouse oocytes. We comprehensively mapped the tRNA landscape from oocyte to blastocyst stages in mice. We observed a distinct transition from oocyte tRNA repertoires to embryonic tRNA pool coded by nuclear and mitochondrial genome, accompanied by a remarkable upregulation of tRNA pseudogenes (tRXs) at 4-cell stage. Integrative multi-omics analyses demonstrated a stage specific transcriptive activation of tRNA genes. Specifically, the activation of zygotic tRNA genes coincides with zygotic genome activation (ZGA) at 2-cell stage, and is burst from 4-cell stage, which correlated with H3K4me3 modification establishment and chromatin state remodeling surrounding tRNA gene loci. Importantly, we also observed an extensive reprogramming and intricate cross- talk of tRNA modifications landscape during embryogenesis. By integrating ORACLE-tRNAseq data with Ribo-seq, we further discovered that embryonic tRNA anticodon pools were preferentially coordinated with high translation-efficiency (TE) gene pools for mRNA processing and translational control, which ensured the rapid establishment of zygotic translation machine after major ZGA. Collectively, decipering the dynamic landscape of embryonic tRNA repertoires provides novel insights to explore the translational regulatory network during early embryogenesis.

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