Microproteins 2023

Poster abstracts

*Session 1 (**bold**: flash talks)*

1. **Marion Martinez, Barcelona**
   Title: *Mining the secreted microproteome for novel regulators of PDAC communication*

   Microproteins bear essential roles in a plethora of biological processes, both in physiology and pathology. In cancer specifically, microproteins have been shown to regulate most tumour hallmarks, and present a huge potential for the clinic as diagnostic and prognostic biomarkers or therapeutic targets. Interestingly, their small size makes them ideal candidates to be shed in tumour-derived exosomes. PDAC-shed exosomes have been shown to prepare the pre-metastatic niche in the liver, and their presence in the bloodstream can be used as a surrogate marker of metastasis.
   Here, we have mined the PDAC exosome-secreted microproteome for novel regulators of tumour progression and metastasis. Using proteogenomics in PDAC patient-derived explants, we have identified 439 microproteins secreted in exosomes by pancreatic tumours. We have selected a set of top microprotein candidates for further characterization by in silico analyses. We have confirmed their exosome-secretion in PDAC cell lines, and preliminary characterisation of these top candidates has shown that they extrinsically promote PDAC cell growth and invasion in vitro. Together, this work advances our knowledge on the underexplored field of secreted microproteins and provides pioneering evidence of their role in tumour cell communication in PDAC. It may further be a source of novel therapeutic targets and PDAC biomarkers for liquid biopsy in the clinic.

2. **Pablo J. Fernandez-Marcos, Madrid**
   Title: *The microproteome of short-term fasting as a guideline for the design of strategies against aging*

   Caloric restriction is the most robust intervention extending healthspan and delaying age-associated pathologies. We use fasting, a type of calorie restriction, as a guide to design new strategies to fight aging-associated pathologies. In particular, we have (1) identified and characterized new molecular pathways affected by fasting, that improve the outcomes of anti-cancer chemotherapy (ChT): the induction of the CDK inhibitor p21, that is necessary for fasting to reduce ChT secondary effects and to improve ChT anti-tumor efficacy; and coordinated changes in the fatty acid profile of cell membranes with fasting. These changes are common between mice and humans, and predict the extent to which fasting protects from ChT toxicity. (2) We have found a new compound (IMDEA C1) that improves mitochondrial function (mitohormesis) and delays aging by modulating the antidepressant targets monoamine oxidase (MAO-B), degrading serotonin; and the GABA receptor GABAAR, specifically in peripheral tissues. Treatment with IMDEA-C1 extended lifespan in worms and flies, improved diabetes in a mouse model of obesity and delayed frailty in very old mice. Taking these findings as starting points, we will join forces with Dr. Maria Abad's laboratory (Altos labs, Cambridge), specialized in microproteins during cellular reprogramming and aging, to better understand and exploit the secreted microproteome during the process of fasting and in the new pathways that we have discovered.

3. **Louise Petri, Copenhagen**
   Title: *Role of microProteins in stem cell maintenance and leaf formation in Arabidopsis thaliana*

   The shoot apical meristem (SAM) is a crucial structure for plant development. The SAM consists of stem cells, which upon differentiation generates the aerial tissue of plants such as stem tissue, leaves and flowers. Differentiation is controlled by transcription factors of the CLASS III HOMEODOMAIN LEUCINE ZIPPERs (HD-ZIP IIIs). HD-ZIPIIIs affects genes involved in leaf polarity and maintenance of the meristem. Among the HD-ZIPIII target genes are the genes encoding LITTLE ZIPPER (ZPR) microProteins. These microProteins feedback-regulate HD-ZIPIIIs. While some loss- and gain-of-function mutants have been studied, it is currently unclear how the loss of all ZPR genes affects stem cell development. We are currently performing a thorough examination of the phenotypes observed in higher-order mutants and also elucidating the expression patterns of all ZPRs . Finally, we are using protein-engineering to decipher the molecular interactions between HD-ZIPIIIs and ZPRs. The latter studies will enable us to precisely alter microprotein function and thereby control stem cell maintenance.

4. **Alessandro Gori, Milano**
   Title: *Reinventing bradykinin function: selective binding to highly curved lipid membranes for extracellular vesicles isolation and analysis*

   Bradykinin (BK) belongs to the family of kinins, key modulators of functions including vasodilation, blood pressure regulation, and inflammation. While these effects are mainly exerted through a paracrine effect on B1 and B2 GPCRs, a growing body of evidence supports the hypothesis of an active role of the lipid membrane as a catalyst for BK–receptor(s) interaction. Within the frame of BK-membrane interplay, intriguingly, multivalent BK constructs were shown to possess a remarkable selectivity towards lipid structures characterized by high membrane curvature. Herein, we turned these BK curvature sensing properties into a radically new use of BK analogues, and specifically their exploitation as affinity probes for the pan-specific isolation of extracellular vesicles (EVs). These nanosized membranous particles released by cells play a pivotal role in intercellular communication, and are increasingly being acknowledged as the next generation theragnostic tools. EVs membrane, given its characteristic high curvature, can be considered as a “universal” EVs marker, oppositely to surface-associated proteins which suffers instead from remarkable heterogeneity. The use of BK derivatives as highly efficient ligands for multipurpose and unbiased EVs isolation shows considerable advantages over the traditional use of antibodies, as here demonstrated by their direct integration onto advanced microanalytic platforms towards liquid biopsy applications.

5. **Maurizio Junior Chiurazzi, Copenhagen**
   Title: *Controlling flowering of Medicago sativa (alfalfa) by engineering dominant microProteins*

   Alfalfa is the most grown forage in the world. It has high yields, good nutritional value, resilience and can fix atmospheric nitrogen. Traditional breeding could only marginally improve alfalfa, as it is a tetraploid and obligate outcrossing plant affected by inbreeding depression. However, inducing dominant mutations to domesticate plants with such complex genomes is an alternative strategy that can be relevant in the control of flowering time, which affects biomass production and forage digestibility. MicroProteins are powerful developmental regulators that can be engineered from existing genes. We are currently conducting proof-of-concept studies in Arabidopsis to evaluate the impact of converting specific genes into microProtein-coding genes, as well as applying this strategy in alfalfa in a dominant fashion.
   For this, a clonal population of the alfalfa Fado variety was established. We re-sequenced and assembled the genome and used transcript-isoform sequencing to identify the most suitable allele(s) for genome-engineering. Current work involves regeneration of genome-edited plants and phenotypic characterization.

6. Celio Dias Santos Junior, Shanghai CANCELLED
   Title: *AMPSphere: Insights about the prokaryotic antimicrobial peptides from the global microbiome*

   Antimicrobial peptides (AMPs) are short sequences (10-100 amino acids) found in all domains of life, which interact with different cell targets (e.g. membranes) to impair microbial growth. AMP applications are vast, e.g. as food preservatives. Despite their critical ecological roles in balancing competition and cooperation among microbes, little is known about the diversity and distribution of AMPs. We focused on ribosomally produced AMPs in 63,410 public metagenomes from 72 distinct habitats and 87,920 high-quality microbial genomes using the program Macrel and generated AMPSphere (https://ampsphere.big-data-biology.org/), a collection of 863,498 non-redundant peptides. Most (91.5%) of AMPSphere is novel, with no homologs in reference databases (e.g. DRAMP and GMGCv1). AMPs' evolutionary history includes processes such as the early termination of longer proteins and gene duplication events. AMPs from AMPSphere are clonal and strain-specific (89% encoded by single or two genetic variants), which might explain the presence of cross-habitat borders (10.8% of multi-habitat AMPs). Within the human body, human mouth bacterial strains presented fewer AMPs, proportionally, than in the human gut. 26 of the 50 peptides tested in vitro demonstrated activity against the ESKAPE group of pathogens. In conclusion, AMPSphere is a resource to understand better AMPs, which may lead to advancements in clinical and industrial operations.

7. **Yiqian Duan, Shanghai**
Title: *With small ORFs come great datasets: the global microbial small ORFs catalog (GMSC)*

Small open reading frames (smORFs) shorter than 100 codons are often neglected due to the limitation of computational and experimental methods. Recently, systematic studies have shown that the small proteins they encode participate in crucial physiological functions. However, little is known about the distribution and role of smORFs in the global microbiome. Here, we constructed the global microbial smORF catalog (GMSC) derived from 63,410 publicly available metagenomes across 72 distinct habitats and 87,920 high-quality isolated microbial genomes. GMSC contains 964,970,496 non-redundant smORFs, the majority of which are novel. Only 8.98% of smORFs have known domains, and 14.06% of smORFs are predicted to be transmembrane or secreted, indicating their potential role in cell communication. We characterize the taxonomy of GMSC, and the densest genera in terms of how many smORFs are found per assembled megabasepair, are Synechococcus, Helicobacter, and Treponema. The smORF density varies by habitat, indicating different prevalence and potential roles of smORFs across habitats. To facilitate the use of this resource, we provide a tool called GMSC-mapper for identifying and annotating the reliable smORFs. In conclusion, the global microbial smORF catalog (GMSC) and the GMSC-mapper tool show an immense and underexplored diversity of smORFs across different habitats and taxonomy, and can be used to research into the presence, distribution and role of smORFs at the global scale.

8. **Oza bin Zaheed Maheswaran, Cork**
Title: *Analysis of Translation of Long Non-coding RNAs*

Proteins are essential for all cellular processes. Ribosomes direct protein synthesis from open reading frames defined by a start and stop codon present in messenger RNAs in the same reading frame. The ability to code for proteins separates messenger RNAs from non-coding RNAs; however, using ribosome profiling, evidence is growing that many transcripts classified as long, non-coding RNAs (transcripts >200 nucleotides), are translated. In our work, we selected several long non-coding RNAs from their differential expression in cancer cell lines and analysed evidence for translation and resultant polypeptide characteristics.
Using Riboseq.Org tools, we visualized ribosomal footprints on transcripts of interest aligned to the reference genome and transcriptome. The sequence of amino acids encoded was then used to assess its protein properties. Characteristics such as codon substitution (comparing to a 24 mammal subset from an alignment of 100 mammals), presence of disordered regions and candidate motifs were scrutinized using the publicly available tools. Lastly, we modelled our candidate microproteins using protein structure prediction methods.
From this, we identified several small open reading frames that encode polypeptides that are less than 100 amino acids (potential microproteins) from long, non-coding RNAs. We anticipate that our work will allow for further discovery of potentially novel bioactive peptides originating from annotated non-coding RNAs.

9. **Lorenzo Lafranchi, Stockholm**
   Title: *Identification of stress-related microproteins*

   Microproteins encoded by short open reading frames (sORFs) of less than 100 codons have been predicted to constitute a substantial fraction of the eukaryotic proteome. However, relevance and roles of the majority of microproteins remain undefined because only a small fraction of these intriguing cellular players have been in-depth characterized so far. Hence, major challenges remain to elucidate which of the thousands of putative translated sORFs are biologically relevant and to functionally characterize their protein products.
   Translational regulation is a central component of stress responses and under stress conditions ribosomes engage with many uncharacterized ORFs. Despite some of these translational events could lack function at the protein level, we can expect various microproteins to be involved in pathways of stress mitigation. To identify stress-related microproteins, we performed phenotypic screens using a custom-made pooled library of putative sORFs. When challenging cells with the nucleotide analogue 6-Thioguanine, we identified two cytoprotective microproteins that we are currently characterizing. Our data show that one of these microproteins is a novel component of the endoplasmic reticulum, where it fulfils its function by interacting and regulating the protein disulfide isomerase ERp44. Besides providing mechanistic insights on a new microprotein, this study highlights the power of using pooled overexpression screens to identify functional microproteins.

10. **Frederik Tidemand, Copenhagen**
    Title: *Largescale production of modified plant peptide hormones: Using E. coli to incorporate non-canonical amino acids*

    Since the discovery of the first plant peptide hormone in the mid 90's, these peptides have made a considerable impact on our perception of inter plant cell signaling. More than 1000 putative peptides have been identified in Arabidopsis with multiple different functions. Many of the identified peptides are characterized by being heavily modified with e.g. tyrosine sulfation, glycosylation and proline hydroxylation. A specific group of peptides essential for root growth are defined by having one or two tyrosine sulfation in their relatively short sequences (between 5 and 18 residues). These sulfations are essential to the function of the peptide but is a major bottleneck in studies of these peptides, as they are very expensive to synthesize. To overcome this issue, we have utilized recent developments in non-canonical amino acid insertion using E. coli. By redirecting the amber stop-codon using a foreign tRNA that is loaded with sulfated tyrosine (sTyr), we ensure specific incorporation of sTyr in the correct position. We are mainly interested in the peptide PSY1, and are now able to produce sTyr containing PSY1 using standard E. coli growth conditions with yields on a scale that far exceeds what is reasonable from chemical synthesis. In addition, our system makes it easy to produce similar peptides, as the E. coli strain developed is generally compatible with sTyr insertion, and thus the sequence of the expression construct could simply be swapped to a different peptide.

11. Ylenia Vittozzi, Copenhagen

Title: *Modulation of tomato growth and development by microprotein-engineering*

Tomato (Solanum lycopersicum L.) is an important food crop and an ideal dicot model for investigating fruit development and yield. MicroProteins (miPs) have recently been shown to be valuable post-translational tools to modify agronomical traits in plants. MiPs are small and contain typically only a single protein-protein interaction (PPI) domain, which is sequence-related to domains found in larger, multi-domain proteins. By interacting with a related protein, miPs can negatively interfere with the formation of complexes involved in biological mechanisms. Thereby miP-interference can lead to improvement of plant breeding and a better understanding of molecular pathways. My study is focused on LITTLE NINJA (LNJ), a NINJA-related miP known to be a master regulator of shoot architecture and compactness in monocots. LNJ is a positive regulator of the Jasmonic Acid (JA) pathway by inhibition of NINJA, which is a co-repressor of early JA-genes. LNJ is not annotated in the tomato genome, but with CRISPR-gene editing we successfully engineered a LNJ miP by genetically modifying the NINJA-like protein AFP3 (ABI5-binding protein 3). AFPs are homologues of NINJA and in A. thaliana they are known to be induced by Abscisic acid (ABA) or abiotic stresses. However, the role of AFP3 in tomato is still unknown. Together with LNJ, AFP3 represents a good candidate to investigate whether LNJ can modulate hormone pathways, and provide a better understanding of its physiological role.

12. Marta Espinosa Camarena, Barcelona

Title: *Long non-coding RNAs and novel transcripts as the principal source of potential novel neoantigens in hepatocellular carcinoma*

Non-canonical peptides derived from the translation of open reading frames (ORFs) in tumor-specific long non-coding RNAs and novel transcripts are a potentially important class of cancer neoantigens, but they remain poorly characterized. These peptides could be more immunogenic than those derived from single mutations and help explain why different patients show very distinct responses to immunotherapy. We have developed a novel computational pipeline to identify putatively translated small ORFs in non-coding and novel transcripts using RNA-Seq and Ribo-Seq data from hepatocellular carcinoma. In order to quantify the number of different types of neoantigens, we have estimated the affinity between cancer-specific peptides and MHC I receptors and investigated cancer immuno-peptidomics data. The results indicate that non-canonical peptides are likely to make a larger contribution to the generation of neoantigens than cancer testis antigens or mutated proteins. One example is LINC01419, a tumor-specific long non-coding RNA that contains several ORFs with translation evidence and which is likely to generate peptides with strong affinity for MHC I. The study shows that non-canonical peptides might have a more important role in mediating cancer immunogenicity than initially anticipated, opening new avenues to develop new anti-cancer treatments such as vaccines.

13. **Patrick Theunissen, Pamplona**
Title: *Microproteins translated from long non-coding RNAs contain epitopes with high immunogenicity and strong potential to be used as vaccines*

Despite the advent of immune checkpoint inhibitors for cancer immunotherapy resulting in improved anti-tumoral activity, a large subset of non-responding patients progresses at a rate consistent with the natural course of the disease. This is especially prevalent in hepatocellular carcinoma (HCC), leading to low survival among patients. With the end of improving patient response and prognosis, we propose a combined therapy using checkpoint inhibitors and immunotherapeutic anti-cancer vaccination. For the development of cancer vaccines, we searched for putative microproteins, according to public RiboSeq data, deriving from long non-coding RNAs (lncRNAs) with high cancer-specific expression. These microproteins could be potential antigens for anticancer vaccination. To prove this hypothesis, we searched in the collection of microproteins for epitopes with high HLA class I/II binding affinity and immunogenicity using in silico studies. The high affinity of the best candidates was also demonstrated in culture and in animal models. Furthermore, we generated alphavirus-based recombinant vectors encoding auto-replicative RNA molecules expressing the antigens of interest and we demonstrated a superior immunogenicity in mice. These results encourage us to develop anticancer vaccines based on lncRNA-derived microproteins for the treatment of patients with HCC and other tumors.

14. Arianne M. Babina, Uppsala CANCELLED
Title: *Investigating the de novo origins of small protein-coding genes: Rescuing an E. coli auxotroph by small proteins selected from random sequence*

Although increasing numbers of small proteins with diverse physiological roles are being identified and characterized in both prokaryotic and eukaryotic systems, the origins and evolution of small protein-coding genes remain unclear. Computational studies in several organisms suggest that many of these small genes may have emerged de novo from previously nonfunctional DNA. However, in vivo experimental data demonstrating this process has so far been limited to selections using structurally-constrained and partially randomized DNA libraries and antibiotic resistance screens. In this work, we selected small proteins (≤50 amino acids in length) from completely randomized sequence libraries that rescue the growth of an Escherichia coli serB deletion auxotroph by up-regulating hisB expression via direct interactions with the his operon mRNA. In addition to their small size and gene regulatory roles, the isolated proteins exhibit other traits characteristic of most known naturally-occurring small proteins, including being primarily hydrophobic and alpha-helical in structure, thus providing further in vivo evidence that small proteins encoding novel beneficial functions can indeed originate de novo from the expression of random and/or nonfunctional DNA sequences. Furthermore, this study adds RNA regulatory elements as another interacting partner for the novel small proteins isolated from our in vivo random DNA library screens.

15. Iñaki Merino Valverde, Barcelona
    Title: *MASALA microprotein: a novel regulator of cellular plasticity encoded by a lncRNA*

    Many assumed non-coding regions, such as lncRNAs, have been demonstrated to actually code for small proteins of less than 100 amino acids. These are called microproteins, and due to their size they had been systematically overlooked. Among these microproteins we aimed to find novel regulators of cellular plasticity and cancer. In this work, we have identified MASALA, a 78-amino acid microprotein encoded in a gene misanotated as a long non-coding RNA. MASALA localizes in the outer mitochondrial membrane and its expression is upregulated upon cytotoxic damage in a p53 dependent manner. Moreover, its expression correlates with a better prognosis in different cancer types and, in fact, it is downregulated in several tumor types, suggesting that MASALA has a tumor suppressor role. In agreement with this hypothesis, overexpression of MASALA impairs the oncogenic transformation of mouse primary fibroblasts, as well as their transformation to induced pluripotent stem cells (iPSCs) through cellular reprogramming.
    Our analyses revealed that MASALA overexpression alters mitochondrial dynamics and reduces mitochondrial respiration. Finally, from the analysis of MASALA interactome we have selected a potential interactor: MARCH5, a E3-ubiquitin ligase which could be modifying the mitochondrial dynamics through MASALA interaction. Summarizing, we identified a novel regulator of the mitochondrial dynamics that acts as a molecular barrier in cellular reprogramming and cancer.

16. Emanuela Greco, Barcelona
    Title: *MIDORI is a novel microprotein that blocks the EMT programme and inhibits breast cancer metastasis*

    Recent findings have revealed that many RNAs previously annotated as non-protein coding actually code for evolutionary conserved microproteins. To date, only a few have been functionally characterised, but they play functions in fundamental processes such as DNA repair and tissue regeneration. This discovery opens a new level of biological complexity, with implications from basic research to the clinical setting. We have identified MIDORI, a microprotein encoded by ZEB2-AS1, a lncRNA dysregulated in many cancers. Our experiments show that MIDORI downregulates the expression of several EMT-related genes and impairs the activation of the TGFβ effectors SMAD2, ERK1/2 and NF-κB. In agreement, MIDORI overexpression in mesenchymal triple negative breast cancer cells increases E-CADHERIN and β-CATENIN cell membrane localisation, reduces cell migration and invasion in vitro, and metastasis in vivo. MIDORI-pull down coupled with mass spectrometry analysis has revealed MYBBP1A, a NF-κB repressor, and HMGA1 as MIDORI interactors. Moreover, we found that MIDORI decreases the secretion of HMGA1 and TGFβ cytokines, suggesting that it may block EMT both at the transcriptional level and by modifying the cell secretome. Altogether, our results indicate that MIDORI is a novel tumour suppressor acting as a negative regulator of EMT, providing proof-of-concept evidence for the relevance of the microproteome in cancer.

17. Jip van Dinter, Utrecht
    Title: *Microproteins as putative therapeutic Targets in Fusion-Positive childhood Rhabdomyosarcoma*

    Rhabdomyosarcoma is a malignant tumor of skeletal myoblast-like cells that mostly occurs in children. It can be classified into two groups: Fusion Negative RMS (fnRMS) and Fusion Positive RMS (fpRMS), most of the latter are categorized as alveolar RMS (ARMS). fpRMS have poor prognostic outcome and are characterized by a gene fusion between FOXO1 and a member of the PAX gene family. Here, we investigate whether these oncogenic fusion proteins, can activate transcription and translation of otherwise silent genomic regions, thereby producing microproteins unique to fpRMS. The translation of normally non-existent transcripts into small proteins may provide a new pool of fpRMS-specific neoantigens targetable by immunotherapy or other therapeutic interventions. To test this hypothesis, we interrogated the transcriptomes of 195 RMS patients and developed a pipeline to detect novel transcripts. This yielded over 4000 putative novel transcripts of which a fraction could only be detected in fpRMS. Over 200 novel transcripts mapped to loci that are not part of existing gene structures. Next, we determined the coding potential of these novel transcripts using RIBO-seq and ORF calling in 4 fpRMS and 4 fnRMS tumoroid models (4 replicates each) as well as 19 primary tumor tissues. Our current efforts are aimed at investigating which transcripts are (i) transcriptionally controlled by the fusion protein, (ii) translated into microproteins, and (iii) presented to the immune system via MHC-I.

18. Luuk Broeils, Utrecht
    Title: *Evolution and implications of de novo genes in humans*

    Genes and translated open reading frames (ORFs) that emerged de novo from previously non-coding sequences provide species with opportunities for adaptation. When aberrantly activated, human-specific de novo genes and ORFs can code for microproteins that have disease-promoting properties, for instance driving tumor growth. Thousands of putative de novo coding sequences have been described in humans, but we still do not know what fraction of those ORFs has readily acquired a function. Through manual curation of literature and databases, we have assembled and re-analysed most de novo genes reported for humans to date. We re-evaluate each locus by tracing the enabling mutations, list proposed disease associations, protein characteristics, and supporting evidence for translation and protein detection. Our list of human de novo genes consists of 82 genes, of which 73 were annotated as lncRNA. However, only 2 of the 82 genes lacked detection of translation. Additionally, we excluded 97 genes found to be de novo in earlier studies, for various reasons including lack of recognition in current Ensembl version, being merged with other gene ids or because their translation initiation site could not be defined. We are currently investigating the abundance and roles of putative human-specific de novo genes and microproteins in pediatric brain tumors and soft tissue sarcomas, to explore whether de novo emerged microproteins play roles in normal human fetal development and childhood cancer.

19. Ho Yan Yeung, Salt Lake City
Title: *Highly stable somatostatin receptor 2-selective peptides from fish-hunting cone snails suppress glucagon secretion in rodents*

Animal venoms are unique source of ion channel drug leads, but their potential for the discovery of novel G protein-coupled receptor (GPCR) ligands has not been comprehensively explored. Here we report the discovery of three short, highly stable somatostatin-like peptides from the fish-hunting cone snails, Conus geographus, which we collectively call the consomatin G1s. Amongst other functions, somatostatin regulates glucose homeostasis by suppressing glucagon secretion via the somatostatin receptor 2 (SST2).
We found that one of the three consomatin G1s, which we term as G1.1, is a highly potent SST2-selective agonist, and displays distinct Gαi/o protein selectivity profile. Given that SST2 plays a vital role in somatostatin-mediated glucagon suppression in the pancreas, we evaluated the glucagonostatic action of consomatin G1.1 by performing static incubation experiments in isolated mouse islets and pancreas perfusion studies in rats. Here, consomatin G1.1 induced a potent glucagonostatic response.
C. geographus is known to utilise insulin to induce hypoglycemic shock in its fish prey. Our results suggest that in combination with insulin, C. geographus uses consomatin G1s to counteract the insulin-mediated increase in glucagon secretion, thereby exacerbating hypoglycaemiac shock in prey. Furthermore, consomatin G1s may serve as valuable source for identifying pharmacological tools that target somatostatin signalling.

20. Amalie Scheel Tost, Copenhagen
Title: *Unravelling the roles and functions of PSY peptides*

Plants have evolved highly specialized cell-to-cell communication to regulate growth and development and to adapt to their surrounding environment. Among the specialized signaling molecules, secreted post-translationally modified peptides are recognized as fundamentally important in plants. The family of plant peptides containing sulfated tyrosine (PSYs) are a group of tyrosine-sulfated peptides, perceived by plasmamembrane-localized leucine-rich repeat receptor kinases (LRR-RKs). The peptide family consists of nine homologs (PSY1-9). All nine PSYs share a highly conserved domain at the c-terminus. The roles and functions of the PSY peptides are difficult to identify as the nine homologs have redundant functions, though their expression pattern is tissue- and even cell specific for some homologs. Despite their highly conserved active domain, the nine homologs do not all share the same post-translational modifications. Recent studies suggest that the PSY homologs are perceived by different receptors, serving different physiological functions. Based on expression analysis, phenotypic studies, reporter lines and recent publications, I will give an overview of the known and proposed roles of PSY peptides and their suggestive functions.

*Session 2 (**bold**: flash talks)*

21. Leron Kok, Utrecht

Title: *Predicting tumor-specific neoantigens for the identification of novel immunotherapeutic targets*

Tumor-specific peptides presented by MHC-I can be targeted by immunotherapy. However, for pediatric cancers, the low burden of somatic mutations makes it difficult to identify such antigens. Non-canonical peptides derived from tumor-specific non-canonical microproteins could serve as an alternative source of tumor-specific neoantigens. Non-canonical microproteins can be inferred from open reading frames (ORFs) predicted from ribosome profiling data. We compared two ORF detection algorithms (ORFquant and PRICE) on high-quality ribosome profiling data of pancreatic cells to determine whether the combination of the two would improve the diversity and comprehensiveness in detected ORFs. PRICE was able to detect over 20,000 ORFs that were not detected by ORFquant, but its specificity was lower. We then used MHCFlurry 2.0 to predict which epitopes may be produced from newly found microproteins. We applied this method to seventeen neuroblastoma-specific ORFs. Ten peptides predicted to be presented by MHC-I were experimentally validated. Five peptides were found to bind significantly better to MHC- I than the negative control. We anticipate that, after further validation, these newly discovered predicted tumor-specific peptides could serve as putative immunotherapeutic targets in the treatment of neuroblastoma. Ultimately, the prediction of non-canonical tumor-specific neoantigens from ribosome profiling data may be used to find immunotherapeutic targets in other pediatric cancers.

22. Damon Hofman, Utrecht

Title: *Identifying microprotein-derived tumor-specific neoantigens in pediatric cancers*

Pediatric cancers often lack targetable somatic mutations, impeding the development of immune therapies. Recent studies have shown that microproteins translated from non-canonical ORFs can contribute to cancer cell survival and populate the cancer immunopeptidome. This suggests non-canonical microproteins can serve as an alternative source of targetable tumor-specific neoantigens. However, their potential as targets for immune therapies in pediatric cancers remains unknown. To investigate this, we developed an integrative transcriptome assembly and ribosome profiling approach to identify small proteins translated from short ORFs unique to different childhood cancers. Using this approach, we systematically interrogated tumor-specific ORF translation in more than 80 patient samples across four types of pediatric cancer. We identified multiple tumor-specific novel microproteins, including a highly expressed microprotein unique to MYCN-amplified neuroblastoma with high MHC-1 binding potential. These results suggest that translation of non-canonical ORFs can be a source of tumor-specific neoantigens, opening up new possibilities for the development of immune therapies against pediatric cancers.

23. Lars Eicholt, Münster

Title: *Structure prediction and analysis of de novo emerged proteins in Drosophila*

How do new genes emerge? It was long thought that new genes can only emerge via duplication and divergence, but novel genes and hence proteins, can also emerge from formerly non-coding DNA; they emerged de novo. Emerged from formerly non-coding DNA, it can be assumed that de novo proteins share more similarities with random sequences than with the majority of known protein families. Contemporary protein families can be seen as island in the ocean of possible sequences. Only a few have surfaced during the course of evolution while most remained submerged or plunged. De novo proteins are novel island distant from the rest in this ocean and could further provide unique structures and folds. Experimental structural determination of de novo proteins can yet only be performed via NMR due to their small size and high disorder content. Additionally, AlphaFold2 seems to reliably predict the structure of de novo proteins while the question remains how AlphaFold2 performs on de novo proteins in comparison to natural language based predictors. Our goal is to use combinations of experimental and computational tools to elucidate the structures of de novo arisen proteins and to further compare those with known protein families and random sequences, thereby opening new directions for protein engineering and expanding our understanding of early protein evolution.

24. Federica Pennisi, Verona

Title: *The role of tomato B-box MicroProteins in reproductive development*

Microproteins (miPs) are single-domain proteins that act as post-translational regulators of protein complexes. In Arabidopsis, two miPs, miP1a and miP1b, are involved in the photoperiodic control of flowering time. These miP1s are members of the BBX family, a group of zinc-finger transcription factors and regulators. miP1a and miP1b actively participate in the Arabidopsis flowering process by mediating the recruitment of CONSTANS (CO) into a repressor complex with TOPLESS (TPL). The interaction of CO with miP1a/b and TPL causes a reduction in the expression of the FLOWERING LOCUS T. SlBBX16 and SlBBX17 are the tomato homologs of miP1b and miP1a, respectively. To study the functional role of these tomato microproteins, we overexpressed them in both tomato (cv MicroTom) and Arabidopsis, focusing on phenotypic analyses during reproductive development. Overexpression of SlBBX16 and SlBBX17 in Arabidopsis had a slight influence on the transition from vegetative to flowering stage. Tomato plants overexpressing SlBBX17 continued to produce flowers for a longer period than wild-type plants. Since there is no evidence in the literature for the presence of an Arabidopsis-like flowering inhibitor complex in tomato, we are testing the interactions of SlBBX16/17 with key known flowering regulators by yeast-two-hybrid analysis.

25. Eilidh Ward, Leeds

Title: *Micro-peptide Discovery: A Comparison of Multiple Ribo-Seq Datasets in Neuronal Differentiation and Development*

~40% of all human lncRNAs are expressed in the brain and exhibit precise spatiotemporal expression profiles. Though many lncRNAs have characterised functions in the nucleus, ~54% of lncRNAs are detected in the cytoplasm, the majority of which are capped and polyadenylated. Many groups have reported the interaction of cytoplasmic lncRNAs with ribosomes, including ours. Some of these ribosome association events result in the translation of small open reading frames (lncRNA-smORFs), with a subset producing stable micro-peptides. Such micro-peptides have been implicated in numerous cellular processes e.g. pTUNAR, a transmembrane micro-peptide involved in neurite formation and a possible activator of SERCA2, but the molecular functions of many remains a mystery. These translated lncRNA-smORFs may represent novel protein coding regions, which may evolve into canonical protein-coding genes.
Our group has previously identified a population of 45 lncRNA-derived micro-peptides, expressed during neuronal differentiation, 36% of which have primate orthologues. Work is underway to determine whether these lncRNA-smORFs are detected in multiple Ribo-Seq datasets and to dissect the molecular mechanisms by which novel micro-peptides function and evolve. The identification of novel neuronal micro-peptides will be important in helping to uncover their fundamental roles during human brain development and neurodegenerative diseases.

26. Ebbe Engholm, Copenhagen

Title: *Development of novel insulins based on the Conus geographus G1 insulin*

Diabetes mellitus is a disease that affects millions of people worldwide. Since its discovery in 1921, insulin has been used for treatment of the disease and many long- and fast-acting insulins have been developed in recent years to help stabilize the blood glucose of diabetes patients. A way of making an ultra-fast-acting insulin could be to prevent the insulin from forming dimers. A challenge of making such an insulin is that many of the residues responsible for forming the dimer, such as the C-terminal of the B-chain, are also important for receptor binding. Inspiration on how to make such an insulin has been found in a very unlikely place: Various members of the cone snail family produce vertebrate-like insulins, which they use to cause hypoglycemia in the fish they hunt. Prior to engulfing and eating the fish, they either inject the fish with insulin by harpooning or introduce the insulin via the gills of the fish simply by secreting the insulin in the water around the fish. Despite lacking the C-terminal of the B-chain, the G1 insulin used by the Conus geographus cone snail is an exclusively monomeric insulin with a potency close to that of human insulin. This makes it a good template for the development of fast-acting insulins. In this study, a number of novel insulins based on the G1 insulin have been synthesized by a multistep solid-phase peptide synthesis (SPPS) approach.

27. Iris Bea Ramiro, Copenhagen

Title: *Somatostatin analog design: Insights from fish-hunting cone snails*

Somatostatin (SS) is a peptide hormone that inhibits the secretion of various hormones, growth factors, and neurotransmitters by activating a family of five G protein-coupled receptors, the SST1-5. We have recently discovered a group of SS-like peptides in the venom of predatory marine cone snails. These peptides, called consomatins, activate the human somatostatin receptors with varying selectivity and potency.
The presented study is based on the activity of Consomatin Ro1 from Conus rolani, a deep-water species from the Philippines that uses an unusual predation strategy to catch fish. Consomatin Ro1 is selective for the SST1 and SST4 with an EC50 of 2.9 µM and 5.1µM, respectively. SS peptide analogs that activate these two subtypes have shown antinociceptive effects in rodent models of pain, such as the small peptide TT-232 and, as we recently showed, also Consomatin Ro1. Here, we design and functionally evaluate a set of Consomatin Ro1 analogs and show that some of these analogs retain the peptide's selectivity profile but have improved potencies. Together, our findings demonstrate that Consomatin Ro1 represents a valuable compound for the design of new SS analogs with biomedical potential.

28. Kevin Heizler, Regensburg

Title: *A microprotein involved in the regulation of mitochondrial functions*

Mitochondria play a crucial role in maintaining cellular metabolism, homeostasis, energy production and stress response, and their dysfunction is often linked to diseases. These processes are composed and tightly regulated by many small proteins. However, even though some mitochondrial microproteins have already been described, the function of many others remains largely unexplored. In an in vivo xenograft mouse model, we identified a long-non-coding-RNA LINC00493 to be differentially expressed in tumors and metastasis. Interestingly, LINC00493 contains a small open reading frame (smORF) which is conserved among mammals and encodes for the 95-amino acid microprotein Small integral membrane protein 26 (Smim26). This single-pass transmembrane protein localizes to the mitochondria and interacts with proteins involved in mitochondria dynamics and small molecule transport. Knockout of Smim26 in HCT116 cells impairs proliferation and mitochondrial respiration. Furthermore, various metabolic changes can be observed in Smim26-depleted cells, including an altered uptake and release of glycolytic metabolites. Taken together, our study suggests Smim26 to be a regulator of various mitochondrial functions.

29. Fabian Hink, Copenhagen

Title: *Peptides with novel helix-inducing N-cap motif disrupt the Mcl-1–BH3 interaction*

In over 60 % of the protein complexes in the protein database, α-helices are involved in protein-protein interactions (PPIs). Many of these PPIs are associated with disease, making them attractive drug targets. One example is myeloid cell leukemia-1 (Mcl-1) that binds an extended α-helix of BH3-only proteins preventing apoptosis. Mcl-1 is overexpressed in several types of cancer supporting the survival of cancer cells. Drug-like compounds that interrupt PPIs are therefore potential therapeutics, but the discovery of small molecule PPI inhibitors has had limited success. An alternative class with drug-like properties are peptides. Peptides can form extensive molecular interfaces and are able to adopt an α-helical conformation. Specific macrocyclization called stapling or capping constraining the confirmation can further improve affinity, stability and cell permeability. Here, we employed the RaPID system, a combination of flexizyme technology and mRNA display, to screen trillions of unique macrocyclic peptides for their ability to bind Mcl-1. We discovered Mcl-1-binding peptides able to outcompete BH3 peptides. These peptides contained a small macrocycle connecting the acetylated N-terminus with cysteine in the 4th position. This motif is an N-cap stabilizing the peptide in a helical confirmation and improved binding affinity. The compatibility with solid-phase peptide synthesis and mRNA display makes it an attractive moiety for future de novo peptide drug discovery efforts.

30. Sven Larsen-Ledet, Copenhagen

Title: *Microproteins to the rescue [of unstable proteins]*

Proteins constitute the main functional units of the cell. However, individual proteins rarely operate in isolation; rather, they interact with other proteins and biomolecules to form macromolecular complexes that mediate virtually all cellular processes like signal transduction pathways, protein degradation, cell cycle regulation, gene expression and DNA repair. In my project, we use multiple orthogonal approaches to thoroughly characterize the interaction between MLH1 and PMS2. The main part of the project is focused on using deep mutational scanning experiments to understand how MLH1 variants affect the heterodimerization with PMS2. In addition to this, we will perform phage-display experiments of purified wild-type MLH1 and selected variants that exhibit reduced interaction with PMS2 in cells. This will be done in collaboration with Ylva Ivarsson at Uppsala University. Specifically, we screen for peptides that bind MLH1 near the interface with PMS2, and thus potentially compete with heterodimer formation. We will potentially find altered peptide binding profiles for variants compared to wild-type MLH1, which could suggest that peptides or microproteins bind and disrupt the heterodimerization between MLH1 and PMS2 in the cell.Furthermore, I am involved in another sub-project in collaboration with Joseph Rogers at the University of Copenhagen. In this project, we aim to find cyclic-peptides that can penetrate the cell membrane and bind and stabilize the metabolic enzyme DHFR. Such cyclic-peptides could potentially be used in treatments of diseases caused by protein destabilization.

31. Ana Pinheiro Lopes, Utrecht

Title: *The human leukocyte antigen (HLA) locus lncRNA HCP5 is translated and modulates immune cell function*

The evolution of the human leukocyte antigen (HLA) locus has helped promote immune adaptation specific to the human species. However, for many recently evolved genes in the HLA locus it remains unknown whether they exert immune related functionality. For example, the HLA locus harbors 72 long noncoding RNAs (lncRNAs) with unknown function, coding potential, or capacity to modulate immune cell functions. Here, we combined RNA-seq and Ribo-seq ORF annotations from human tissues and cell lines to catalog HLA lncRNA ORFs. We found translation evidence of 18 lncRNA-ORFs for 7 out of 72 lncRNAs and observed that the majority of these lncRNA-ORFs emerged de novo during primate evolution. Silencing of one translated lncRNA, HLA complex P5 (HCP5), in immune cells impacted their activation and chemokine production which can possibly contribute to tumor evasion. The precise roles of the transcribed HCP5 RNA versus the translated ORF are still being investigated. We hypothesize that the translation of lncRNAs in the HLA locus has co-evolved with immune system adaptation and may therefore unveil novel, potentially human-specific regulatory mechanisms of immune cell functions in cancer.

32. Andreas Kosteletos, Leeds

Title: *Investigating the functional role of translated open reading frames in long non-coding RNAs during neuronal differentiation*

Long non-coding RNAs (lncRNAs) account for ~30% of human genes and ~40% are specifically expressed in the brain. Their precise spatiotemporal expression patterns and dysregulation in neuronal disease suggests physiological and pathological relevance. Moreover, ribosome-profiling and proteomics indicate that many cytoplasmic lncRNAs contain small ORFs (sORF) that translate into microproteins. We have previously performed Poly-Ribo-Seq on a human neuroblastoma cell line, SH-SY5Y, 45 translated sORFs from 35 annotated lncRNAs were detected and exhibited comparative translational efficiencies to protein-coding ORFs. Analysis of public datasets revealed that 39% of the translated lncRNA genes show developmentally dynamic expression and 67% are dysregulated in CNS cancers. To dissect the function of peptides translated from these lncRNAs, 13 candidates were selected based on their conservation and association with development or disease. FLAG-tagged reporter assays demonstrate that 11/13 sORFs produced stable peptides with 6/11 adopting subcellular localisations indicative of function. siRNA knockdown of 3 translated lncRNAs results dysregulated SH-SY5Y differentiation, indicating multiple translated lncRNAs are required in early neuronal differentiation. 2/3 of these translated lncRNAs show dynamic expression during early cortical organoid development. CRISPR experiments are underway to determine whether observed phenotypes are mediated by the lncRNA or its peptide product.

33. Mark Nasef Ragheb, Stanford

Title: *Mining the human gut microbial microproteome for modulators of inflammation*

Vertebrates and their microbiomes have co-evolved for ~30M years, thus secreted microbiome microproteins likely evolved to communicate with the host. Recently, the Bhatt lab discovered >4,500 new microbial microprotein families, a portion of which are likely secreted and may participate in microbe-host crosstalk. Still, the functions of most of these microproteins are unknown. We hypothesize that secreted peptides produced by the microbiome have evolved to signal to human cells, given the co-evolution of many gut microbes with vertebrates. Many of these microproteins may have immunomodulatory functions, and we seek to discover these "cross-talk" signals between microbes and humans.

To test this hypothesis, we will express libraries of micropeptides in macrophages, and measure their functional consequences using a panel of assays. Specifically, we will quantify

the ability of expressed micropeptides to induce M1 vs. M2 macrophage polarization. We will also perform Multi-omic analysis of macrophages in response to micropeptides. We anticipate these studies will provide a rich, new resource of microbial peptide classes that modulate host biology.

34. Jing Zhao, Copenhagen

Title: *Discovery of de novo molecular glues*

Interactions between proteins are crucial in the construction and maintenance of the cell. The network of protein-protein interactions (PPIs) in the human cell is vast and many of these are implicated in disease. Inhibiting PPIs is a possible drug mechanism, however, strengthening, or even creating interactions between proteins has great potential for therapeutic applications. We propose to create a new avenue of drug discovery, by developing and applying RaPID system to find de novo molecular glues: never-seen-before molecules that can strengthen or create interactions between proteins.

35. Yong Zhang, Copenhagen
Title: *Phage encoded micro-proteins in hijacking host bacterial cell*

Phages are the viruses that infecting specifically bacterial host cells. A significant portion of phage genome encode micro proteins below 150aa. However, the majority of these micro proteins remain functionally unknown and unstudied. We recently studied the E. coli phage T7 genome and discovered a few microproteins (varying from 30-123 aa) that potentially interfere the host cell stress response pathways, as a manner to hijack host cell for a successful infection. These preliminary discoveries will be presented and discussed.

36. Erik Abner, Tartu
Title: *Uncovering pathogenic micropeptides from the human genome*

A recent estimate suggests that 7,264 micropeptide-encoding regions are located within the human genome. These protein products are hypothesized to participate in numerous molecular, cellular and physiological processes, yet the function of but a few micropeptides has been identified.
The Estonian Biobank (EstBB) is a population-based biobank, containing samples from around 20% of the adult population from all demographic distributions within Estonia. All the blood samples from the biobank participants have undergone genotyping and are periodically linked to national electronic health records.
This project aims to identify relevant micropeptide-encoding regions from the human genome and characterize the functionality of clinically significant novel protein candidates. Utilizing the genomic and biometric data from EstBB, we are currently carrying out over 7,000 genome-wide association studies with clinical traits, which will allow us to assess the effect genetic variants within the micropeptide-encoding regions have on disease etiologies. Follow-up in silico analyses of resulting micropeptides will concentrate on the sequence homology, structure and specific disease molecular biology to understand the functionality of said proteins.
The identification of novel pathogenic micro-genes and the improved understanding of human micropeptidome will assist in the advancement of clinical research, pharmacology, diagnostic and personalized medicine.

37. Aleksandra Panfilova, Copenhagen
Title: *Predicting the effect of point mutations on protein interactions*

Most processes in molecular biology involve protein interactions. The affinity and specificity of binding to a substrate, a cofactor, or a target molecule are key factors that determine protein function. The activity of proteins is often regulated by small molecules or other proteins, including microproteins, which have been shown to act as protein activity regulators in some cases.
Missense mutations that alter protein interactions can have various effects, ranging from reducing the efficiency of an enzyme to causing a complete loss of interaction with a substrate and resulting in a loss-of-function phenotype. Missense mutations can also destabilize the protein structure, leading to the same loss-of-function phenotype. The prediction of the effect of a specific variant on stability and interactions could help diagnose and treat genetic diseases by providing insight into the mechanism behind the loss-of-function phenotype. Computational tools for such predictions can be especially efficient when applied to microprotein complex structures, as the size of the protein and its interface is a limiting factor for most calculations on protein structures. With AlphaFold2 enabling the prediction of protein tertiary structure, as well as the structure of protein complexes, these predictions can also be obtained for microproteins without experimentally determined structures.

38. Joshua Torres, Copenhagen
Title: *Posttranslational modification (PTM) enzymes in contoxin biosynthesis*

Conotoxins are an important class of peptides that inspired the design of many pharmacological tools and life-saving drugs used today. The structural confirmations that underlie the basis of their exquisite receptor binding properties are partly due to the diversity of posttranslational modifications (PTMs) that they contain. In this work, we identified the PTM enzymes responsible for the bromination, glycosylation and sulfotyrosine transfer in conotoxins. We show, in vitro, how these reactions are performed by these enzymes and compared them with similar enzymes from bacteria, fungi, and plants. Furthermore, we demonstrate the biotechnological potential of these cone snail PTMs to install modifications to synthesize conotoxins and other mollusk derived peptides.

39. Anna Spinner, Stockholm
Title: *Identification of microproteins controlling pluripotency and differentiation*

Mounting evidence indicates that microproteins encoded by short open reading frames (sORFs) are abundantly translated in eukaryotic cells, yet their function remains largely unknown. Therefore, large-scale efforts are crucial to understand how many of the putatively expressed microproteins perform a discernible function in the cell. Due to their remarkable feature to self-renew and differentiate into complex functional tissues, pluripotent stem cells emerge as transformative tool within biomedical research and regenerative therapies. Given the very versatile functions of microproteins, including prominent roles in development, it is not far-fetched to speculate that a subset of microproteins control pluripotency or lineage choice. Consequently, we aimed to identify microproteins functional in pluripotency and differentiation through pooled overexpression screens in human induced pluripotent stem cells and progenitors of neural stem cells. We screened our custom-designed library of putative sORFs using phenotypic selection and fluorescence sorting for surface markers, which yielded several candidate microproteins that are further characterized at this stage. This study illustrates the importance of unbiasedly exploring the true extent of microprotein biology to advance our overall understanding of cellular functioning.

40. Bar Edri, Beer Sheva
Title: *The role of the micro-protein TP73-AS1 in Glioblastoma*

Glioblastoma multiform (GBM), the most common brain tumor, is characterized by a dismal prognosis. GBM cancer stem cells (gCSC) or tumor-initiating cells are the tumor cells that drive therapy resistance and recurrence. While temozolomide (TMZ), an alkylating agent, constitutes the first-line chemotherapeutic - significantly improving survival in GBM patients - resistance commonly leads to GBM recurrence and treatment failure. Recent studies in Professor Barak Rotblat lab suggest that TP73-AS1 lncRNA promotes tumor aggressiveness and TMZ resistance in gCSC. Moreover, the results suggest that TP73-AS1 has both a pathological function and a physiological function in aging. Recent studies have revealed open reading frames (ORF) in the non-coding transcriptome code for small proteins, a.k.a. micro proteins. Functional characterization of a small number of these new micro-proteins demonstrates their critical roles in several biological processes, bringing forth micro-proteins as new and exciting factors in biology. By mining published data, we found that TP73-AS1 encodes two ORF, one of which encodes a 72 amino acid protein - raising the possibility that the functions attributed to the lncRNA TP73-AS1 might be related to the micro protein encoded by the transcript, rather than the transcript itself. This emphasizes the importance of studying the non-coding genome, and demonstrates the opportunities it holds in discovering biological markers and new drug targets.

41. Chris Papadopoulos, Barcelona
Title: *The Ribosome Profiling landscape of yeast reveals a high diversity in pervasive translation*

Pervasive translation is a widespread phenomenon that plays an important role in de novo gene birth; however, its underlying mechanisms remain unclear. Based on multiple Ribosome Profiling datasets, we investigated the Ribosome Profiling landscape of coding and noncoding regions of yeast. Therefore, we developed a new representation framework which allows the visual and comprehensive representation of the diversity of translation signals in yeast coding and noncoding regions. We show that if coding regions are restricted to specific regions of the Ribosome Profiling landscape, noncoding regions are associated with a wide diversity of translation signals. In particular, we reveal that noncoding regions are associated with canonical translation signals but also with novel categories of translation events absent from coding regions, and which seem to be a hallmark of pervasive translation. Notably, we report thousands of translated noncoding ORFs among which, hundreds led to detectable products with Mass Spectrometry. Finally, we show that the translation behavior of noncoding ORFs is not explained by features related to the emergence of function, but is rather determined by the translation start codon and the codon distribution in the three competing RNA frames. Overall, our results enable us to propose a topology of the pervasive translation landscape of a species, and open the way to future comparative analyses of this translation landscape under different conditions.

42. Thomas Lund Koch, Salt Lake City
Title: *Recruitment strategies of signaling peptides to doppelganger toxins in cone snails*

Peptide toxins play a key role in the chemical warfare of the animal kingdom, allowing predators to incapacitate prey and defend against enemies. Understanding the evolution and diversity of toxins can provide insights into both predator-prey interactions and potential therapeutic applications. One class of toxins, known as doppelganger toxins, have evolved to mimic prey signaling peptides to disrupting the prey's physiology. Marine cone snails are an important source of these toxins; however, the full repertoire of cone snail doppelganger toxins is unknown. From a comprehensive transcriptomic and genomic annotation of cone snail signaling peptides, we identify six previously undescribed doppelganger toxin families mimicking NPF, Calcitonin, Egg-laying hormone, NKY, GGNamide, and HFAamide. These toxins all evolved from the cone snail's endogenous signaling peptides, but different molecular phenomenon led to their recruited to the venom, including duplication and neofunctionalization, moonlighting, and exon shuffling. NPF doppelganger toxins provide a unique case study where separate recruitment events have formed three distinct families of toxins. This work sheds light on the diversity of doppelganger toxins in cone snails and highlight the importance of prey signaling peptide mimicry in the evolution of venom systems, with potential implications for drug discovery and understanding the evolution of predator-prey interactions.

43. Lena Ho, Singapore.

Title: *A crispr screen targeting the microproteome uncovers CRISTA – a microprotein that controls mitochondrial translation via one-carbon metabolism*

Mitochondrial small-ORF encoded microproteins (SEPs) are key regulators and components of the electron transport chain (ETC). ETC assembly – particularly that of Complex I - is a costly bioprocess that is tightly coupled to nutrient availability and redox status. Serine is a major source of 1-carbon (1C) units for anabolic growth and redox buffering, and its availability is known to regulate Complex I biogenesis. How mitochondria actively sense serine flux to coordinate Complex I assembly is unclear. Using a genome-wide CRISPR screen against a comprehensive collection of SEPs, we find that lncRNA-encoded microprotein CRISTA is required for protection against 1-carbon stress under oxidative conditions. CRISTA protein levels respond to levels of the 1-carbon pathway to potentiate serine flux via direct N-terminal interactions with SFXN1 in the inner mitochondrial membrane. The C-terminus of CRISTA furthermore interacts with the mitochondrial ribosome to form a trimolecular SFXN1-CRISTA-mitoribosome complex that is dedicated to the translation of Complex I subunits ND5/6. Loss of CRISTA disrupts the trimolecular interaction, leading to reduced mitochondrial serine uptake and downstream transulfuration, resulting in taurine deficiency. These metabolomic deficiencies impair the 5-taurino-methyl-uridinylation of mitochondrial tRNAs that are required selectively for the translation of Complex I ND5/6. Consequently, CRISTA mutants have Complex I translation and assembly defects and are impaired in oxidative metabolism. In mice, loss of CRISTA causes post-implantation lethality that is partially rescued by taurine supplementation. Our work uncovers a novel and essential mechanism that bridges 1-carbon metabolism to Complex I biogenesis. We propose that by apposing a metabolite transporter with the mitochondrial ribosome, the microprotein CRISTA helps to establish a localized domain of metabolites that directly control the rate of mitochondrial translation.