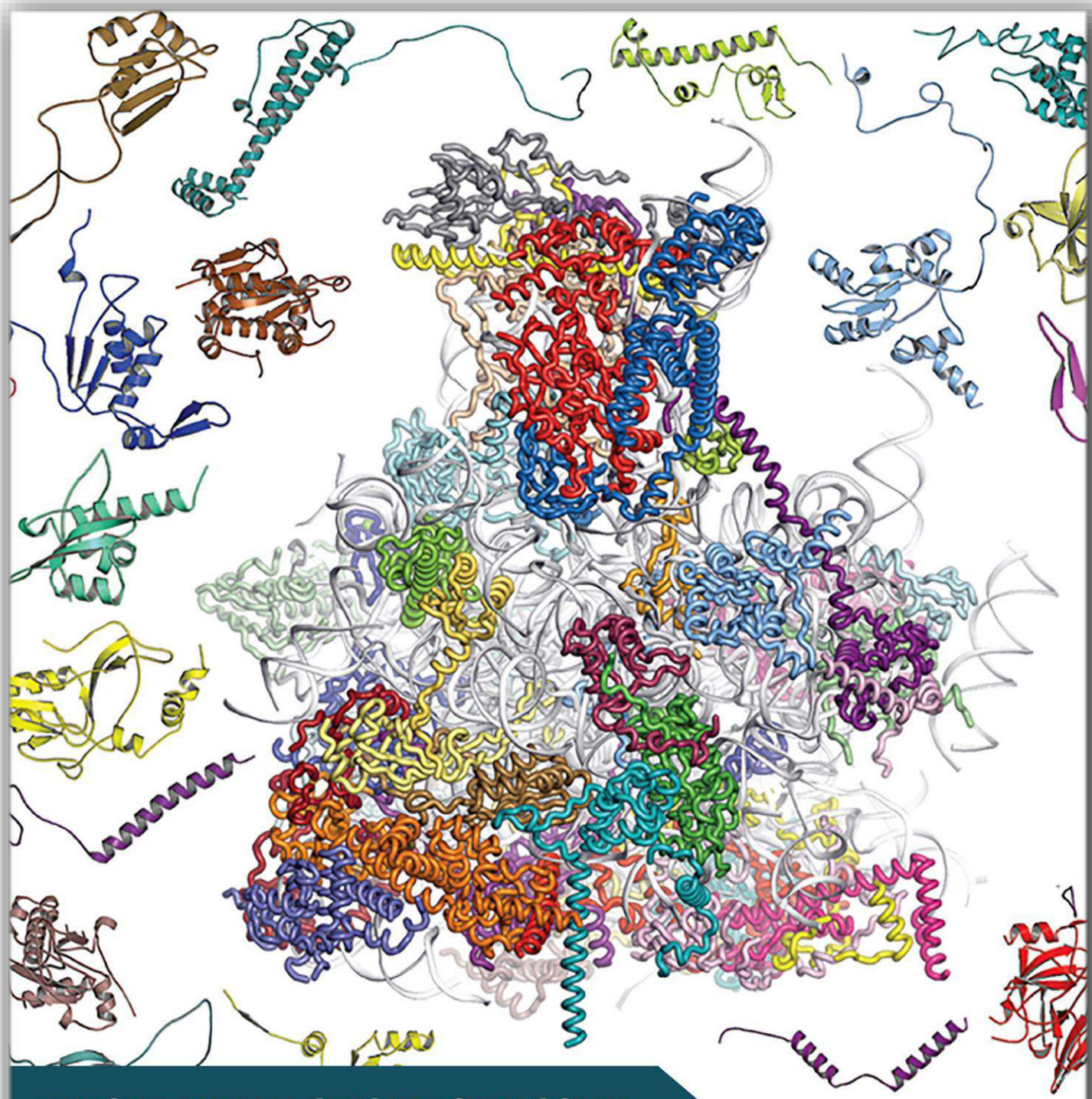

THE 2ND ANNUAL CRYONET SYMPOSIUM

Postrevolution Cryo-EM:
Reaping the benefits while looking to the future

ABSTRACT BOOK

Date: October 10-11
Location: Biomedicum Lecture Hall
Organizers: Alexey Amunts, Bridget Carragher, Ausra Domanska, Gunnar von Heijne

WELCOME TO CRYONET IN STOCKHOLM 2019!

Dear colleagues,

CryoNet is a Swedish-Danish network aiming to promote a strong cryo-EM research environment in the Nordic countries. CryoNet is supported by the Knut and Alice Wallenberg Foundation in Sweden and the Novo Nordisk Foundation in Denmark.

The first CryoNet symposium was held in Copenhagen in 2018. The 2019 CryoNet symposium will cover methodological advances and recent hot structures in 24 talks and 44 poster presentations.

We are looking forward to fruitful discussions and wish all participants an inspiring and memorable stay in Stockholm!!

Sincerely,

Your organizing committee

Knut och Alice Wallenbergs Stiftelse

novo nordisk fonden

Stockholm University

SciLifeLab

CONFERENCE INFORMATION

VENUE: Biomedicum building, Karolinska Institute, Solnavägen 9.

~160 participants registered to the meeting, and the lecture hall has 205 seats and 5 wheelchair spots.

TALKS: Invited speakers – 35 min including questions. Selected students and postdocs – 20 min including questions. Industry – 10 min.

There are 3 wireless headset microphones, and every speaker can have one prepared in advance.

POSTERS: The posters can be set up in the morning or during the coffee break and removed in the evening. The sessions will take place 12.00-13.30. Poster stands will be indicated with presenter's name.

WIFI: via "eduroam". For participants without eduroam access, please use network KI-Guest, password: Stockholm18.

TWITTER: Please use #CryoNet and handles @scilifelab_BMI @Stockholm_Uni

CERTIFICATE: If you would like to receive a Certificate of Attendance, please signal to Vasilis.

CONTENTS

SCHEDULE

Day 1, October 10: Hot structures

09.20-09.30 **Gunnar von Heijne, Stockholm University, Director of SciLifeLab National CryoEM facility**

Opening and Welcome to the 2nd Annual CryoNet Symposium

09.30-10.05 **John Rubinstein, University of Toronto**

Proton pumping V-type ATPases

10.05-10.25 **Alexander Mühleip, Stockholm University**

Structure of a mitochondrial ATP synthase

10.25-10.45 **Ben Falcon, MRC Laboratory of Molecular Biology**

Conformers of assembled Tau in human neurodegenerative diseases

10.45-11.00 Coffee

11.00-11.35 **Kelly Nguyen, MRC Laboratory of Molecular Biology**

Replenishing the ends: structural mechanism of human telomerase

11.35-11.55 **Simone Cavadini, Friedrich Miescher Institute**

DNA damage detection in nucleosomes involves DNA register shifting

12.00-13.30 **Lunch + Poster Session**

13.30-14.05 **Sarah Butcher, University of Helsinki**

Antivirals – a new approach to an old problem

14.05-14.25 **Anna Munke, Uppsala University**

Structure of a marine algae virus, and the evolution of Picornaviridae viruses

14.25-14.45 **Pavel Plevka, Masaryk University**

Structure and genome ejection mechanism of S.aureus phage P68

14.45-15.10 Coffee

15.10-15.45 **Lars-Anders Carlson, Umeå University**

In situ studies of positive-sense RNA virus replication with cryo-ET

15.45-16.20 **Clemens Plaschka, Research Institute of Molecular Pathology**

Structural studies of nuclear pre-mRNA splicing

16.25-16.35 **Adrianna Parizatto, Thermo Fisher Scientific**

Cryo-EM adoption – Krios G4

16.35-16.45 **René Henderikx, CryoSol World**

VitroJet: bridging the gap in sample prep

16.45-16.55 **Michelle Darrow - TTP LabTech**

Chameleon: next generation sample preparation for cryoEM based on Spotiton

Day 2, October 11: New methods

09.30-10.05 **John Briggs, MRC Laboratory of Molecular Biology**

Studying enveloped virus structure using subtomogram averaging

10.05-10.25 **Ahmad Jomaa, ETH Zurich**

Cryo-EM reveals the mechanism of SecA-mediated co-translational targeting of membrane proteins in bacteria

10.25-10.45 **Jana Skerlova, Stockholm University**

Structure of the minimal progenitor toxin complex of botulinum neurotoxin X

10.45-11.00 Coffee

11.00-11.35 **Julia Mahamid, European Molecular Biology Laboratory**

From translational stress response in eukaryotes to transcription-translation coupling in bacteria: Unique insights from in-cell cryo-electron tomography

11.35-11.55 **Maxim Armstrong, University of California, Berkeley**

Micro-scale fluid behaviour during cryo-EM sample blotting

12.00-13.30 Lunch + Poster Session

13.30-14.05 **Johanna Höög, University of Gothenburg**

TAILS of a human sperm

14.05-14.25 **Urška Rovšnik, Stockholm University**

Characterizing functional states of a model ligand-gated ion channel by cryo-EM

14.25-14.45 **Andrii Iudin, EMBL-EBI**

EMPIAR: recent developments and outlook

14.45-15.10 Coffee

15.10-15.45 **Elizabeth Wright, University of Wisconsin–Madison**

Using Cryo-Electron tomography to study host-pathogen interactions

15.45-16.20 **Sharon Wolf, Weizmann Institute of Science**

Chemical and structural insights with cryoSTEM tomography

16.20-16.55 **Peijun Zhang, University of Oxford**

Imaging virus assemblies in situ with cryo-EM

POSTER SESSIONS

Day 1 (October 10th): 12.00-13.30

Perez Boerema A.	Stockholm University	*Structural diversity of photosystem I*
Carlstrom A.	Stockholm University	*TBA*
Forsberg B.	Stockholm University	*Unexpected features and stoichiometric symmetries in the fungal pyruvate dehydrogenase complex*
Larsson D.	Uppsala University	*Structure of an E. coli 50S ribosome assembly intermediate, the substrate of methyltransferase RlmF*
Zyla D.	ETH Zurich	*The assembly platform FimD is essential for the formation of the most stable quaternary structure of type 1 pilus rods*
Iakovleva I.	Umeå University	*Uncovering the amyloid fibrils structure of Transthyretin using cryo-EM*
Berndtsson J.	Stockholm University	*TBA*
Zhang J.	Umeå University	*Observation of TBE Virus replication in mouse brain by electron tomography*
Zhao J.	Stockholm University	*A simple pressure-assisted method for cryo-EM specimen preparation*
Cerny J.	CEIT Brno	*Better structures of nucleic acids with dnatco.org*
de la Rosa Trevin J.M.	Stockholm University	*Pushing the limits of automation for cryo-EM on-the-fly data processing*
Lyons J.A.	Aarhus University	*Structural insights into the function and auto-regulation of lipid flippases.*
Nissen J.D.	Aarhus University	*Investigating the ultrastructure of the axon initial segment by cryo-electron tomography*
Sandblad L.	Umeå University	*Focused Ion Beam methods for Electron Tomography*
Choi J.	University of Oxford, UK	*Towards cryo-EM structure of long non-coding RNA*
Jussupow A.	Technical University of Munich (TUM)	*The impact of cardiolipin on the structure, dynamics and activity of complex I*
Noreng S.	Oregon Health and Science University	*Structure of the epithelial sodium channel by cryo-EM*
Szodorai E.	Karolinska institute	*Secretagogin interactome conveys calcium signaling of insulin release, β-cell identity and survival*
Di Luca A.	Technical University of Munich (TUM)	*Global motions and loop regions are involved in the deactivation of complex I*
Murina V.	Umeå University	*Molecular mechanism of antibiotic resistance mediated by ABCF ATPases*
Happonen L.	Lund University	*Structural biology and structural mass spectrometry in host pathogen protein-protein interactions*
Mühlbauer M.	Technical University of Munich (TUM)	*Charge-transfer dynamics in the membrane domain of respiratory complex I*

Day 2 (October 11th): 12.00-13.30

Siborova M.	CEIT Brno	*Structure of bacteriophage SU10*
Clabbers M.	Stockholm University	*Structure of a novel R2lox protein revealed by micro-crystal electron diffraction*
De Val N.	EML Maryland	*Using electron microscopy to enhance the knowledge of biological systems*
Bardy P.	CEIT Brno	*Structure and DNA delivery mechanism of Rhodobacter capsulatus gene transfer agent*
Nissen P.	Aarhus University	*Single-particle cryo-EM studies of P4-ATPase lipid flippases*
Flygaard R.	Stockholm University	*ATP synthase in Toxoplasma shapes the mitochondria through previously unseen high oligomeric states*
Matsuoka R.	Stockholm University	*Structural basis for Na+/H+ anti-transportation mechanism of NHA2 in SLC9B family*
Righetto R.	Biozentrum Basel	*Cryo-EM of self-assembled FAK 2D crystals reveals domain rearrangements and oligomerization upon membrane binding*
Darrow M.	Sloan Kettering	*Cryo-EM structures of fungal and metazoan mitochondrial calcium uniporters*
Shahzad S.	Umeå University	*Cryo-EM structure of mitochondrial lon protease*
Flores S.	Stockholm University	*Fast multiscale methods extract more information from mixed-resolution density maps of ribonucleoprotein complexes*
Dahmane S.	Umeå University	*In situ structural studies of enterovirus replication using FIB milling and cryo-electron tomography*
Amstrup S.	Aarhus University	*Investigation of phosphonate degradation by the carbon-phosphorus lyase complex*
Rocha de Moura T.	KTH	*Structural studies of the Pannexin 1 membrane channel*
Laurent T.	Umeå University	*In situ studies of alphavirus replication complexes by Cryo-electron tomography*
Tobiasson V.	Stockholm University	*The structure of the mitochondrial ribosome from T.thermophila*
Singh V.	Stockholm University	*Atomic model of human mitoribosome*
Itoh Y.	Stockholm University	*Discovery of novel universal translation factor by cryo-EM*
Wang Z.	KTH	*Visualizing of PICK1 complexes at the membrane interface by Cryo-electron microscopy*
Venkatesha Murthy A.	KTH	*Membrane scaffolds in synapses: towards novel therapy against neurological disorders*
Pulk A, Kipper K.	University of Tartu	*Regulation of localized protein synthesis by neuronal RNA granules*
Kjeldsen R.	Aarhus University	*IgE - The rigid antibody*

INVITED SPEAKERS

John Briggs
MRC Laboratory of Molecular Biology

STUDYING ENVELOPED VIRUS STRUCTURE USING SUBTOMOGRAM AVERAGING

The combination of cryo-electron tomography and subtomogram averaging can be used to determine the structures of proteins within heterogeneous environments. We have applied this approach to obtain insights into the assembly and structure of enveloped viruses including HIV-1. By obtaining structures of proteins “in situ” within viral particles, we can understand how they assemble and rearrange to form infectious particles. I will describe recent data, the techniques used to obtain them, and their implications for understanding viral lifecycles.

Sarah Butcher
University of Helsinki

ANTIVIRALS –A NEW APPROACH TO AN OLD PROBLEM

Picornaviruses include rhinoviruses and enteroviruses. Rhinoviruses cause millions of cases of upper respiratory infections ("colds") yearly and contribute to asthma, and enteroviruses are responsible for millions of infections including cases such as meningitis, encephalitis and polio. There are currently no antivirals that can be used for the treatment or prevention of any of the rhino- or enteroviruses. To replicate, viruses must interact with host cells, and in doing so, often need to change shape; stabilizing the virus particle is therefore thought to be a promising strategy for preventing replication. In a search for potential antiviral candidates, we have found a compound that stabilized a model picornavirus. We performed cryo-electron microscopy (cryo-EM) of the drug-virus complex to determine how the drug exerted its effect. Cryo-EM involves combining thousands of two-dimensional images to develop a highly detailed three-dimensional structure of the target. Although picornaviruses have been studied for decades, we discovered a previously unknown pocket, or indentation, on the surface of the virus, in which the compound had lodged, thereby stabilizing it against the kind of shape change that would allow interaction with host cells. We then used the compound as a starting point to generate multiple variants of the antiviral molecule to maximize the activity against a broad range of picornaviruses. A major challenge in developing antiviral medications is that viruses mutate quickly, changing in ways that make a once-useful drug ineffective. While it is possible that the newly-discovered pocket may also mutate to make picornaviruses resistant to therapies developed against them, we showed that the pocket is crucial enough for viral replication that viruses containing mutant versions are less viable, making the drug relatively "resistance-proof." Further work to develop these compounds into effective drugs is ongoing. These results open up a new avenue for the design of broad-spectrum antivirals against rhinoviruses and enteroviruses, both of which are major human pathogens.

Lars-Anders Carlson
Dept of Medical Biochemistry and Biophysics, Wallenberg Centre for Molecular Medicine, Umeå University

IN SITU STRUCTURAL STUDIES OF POSITIVE-SENSE RNA VIRUS REPLICATION USING CRYO-ELECTRON TOMOGRAPHY

Viruses of the positive-sense RNA ((+)ssRNA) type are a major class of human pathogens causing diseases ranging from common cold to Hepatitis C and arthropod-borne fevers. The extracellular stage of these pathogens, the virus particles, has been studied in great detail often resulting in 3D structures at atomic resolution. The intracellular manifestations of (+)ssRNA viruses - membrane-bound genome replication complexes - have been more recalcitrant to structural studies. Here, I will present our preliminary studies of replication complexes of (+)ssRNA viruses using focussed ion beam milling and cryo-electron tomography of infected cells and tissues.

Johanna Höög
University of Gothenburg

TAILS OF A HUMAN SPERM

Flagella, such as the human sperm tail, are complex molecular machines that consists of up to 1000 different proteins. Without their proper function none of us would exist, yet, human flagella structure is not well studied.

Our cryo-electron tomography study of human spermatozoa, the first of its kind, found that inside the lumen of microtubules a complex structure spanned over several micrometers in the spermatozoon end piece. This structure forms an interrupted helix and binds to the inside surface of the tubulin heterodimer, the protein that forms microtubules. This structure has not been seen in other flagella, which suggests that it might be spermatozoa specific. We named it terminal axoneme intra-lumenal spiral, or *TAILS* for short. The discovery of this novel structure shows the need to study human flagella directly, to understand the components involved in spermatozoon swimming.

TAILS might be involved in stabilization of the microtubules which otherwise are constantly growing and shrinking, or it might make the end piece more rigid which would yield more force in the flagellar beat. We are currently using several approaches to identify the protein(s) that form *TAILS,* which is the first crucial step towards elucidating its function in human spermatozoa.

Julia Mahamid
European Molecular Biology Laboratory

FROM TRANSLATIONAL STRESS RESPONSE IN EUKARYOTES TO TRANSCRIPTION-TRANSLATION COUPLING IN BACTERIA: UNIQUE INSIGHTS FROM IN-CELL CRYO-ELECTRON TOMOGRAPHY

Cells organize their biochemical reactions into functionally distinct compartments. Intriguingly, many, if not most, eukaryotic cellular compartments are not membrane enclosed; they rather assemble dynamically by phase separation. Despite significant progress on reconstituting such liquid-like assemblies in vitro, we lack information as to whether these compartments in vivo are indeed amorphous liquids, or whether they exhibit structural features such as gels or fibers. We develop and employ advanced sample preparation techniques for *in-cell* cryo-electron tomography (cryo-ET), including cryo-focused ion beam thinning guided by 3D correlative fluorescence microscopy. Preparations of site-specific 'electron-transparent windows' in suitable cellular model systems enable direct examination of the structural features of compartments at molecular resolution in their native context. Here, we use these techniques to elucidate the structural principles and cytoplasmic environment driving the dynamic assembly of stress granules, which are ribonucleoprotein granules that form rapidly in the cytoplasm upon cellular stress. Under either heat- or arsenite-induced stress, we observe abundant filamentous assemblies in stress granules inside HeLa cells. The 3D molecular structure of the filaments obtained by subtomogram averaging appears to be distinctly different from amyloid-like fibrils that have been classically associated with proteins engaged in stress granules. Yet, these unprecedented data further illustrate challenges currently hindering in-cell structural biology: (i) the difficulty in assigning a molecular identity to analysed structures, and (ii) a resolution limit in the range of 1 nm. Using a simple bacterial model system, *Mycoplasma pneumoniae*, we demonstrate the potential synergistic application of cryo-ET and in-cell cross-linking mass spectrometry to reveal and interpret 3D macromolecular structures directly inside cells at the secondary structure level of detail.

Kelly Nguyen
MRC-Laboratory of Molecular Biology

REPLENISHING THE ENDS: STRUCTURAL MECHANISM OF HUMAN TELOMERASE

In eukaryotes, linear chromosome ends are capped with telomeres, which protect the chromosomes against end-joining and end-replication issues. Telomeres are also progressively shortened because of incomplete replication by the replisome. Critically short telomere length leads to chromosomal degradation and apoptosis. To balance this loss, telomerase ribonucleoprotein (RNP) synthesizes telomeric repeats through its telomerase reverse transcriptase (TERT) and an integral RNA subunit (hTR) carrying the template for repeat synthesis. In addition to TERT and hTR, the human telomerase holoenzyme consists of a number of other protein factors required for RNP assembly and localization. Telomerase has been shown to play important roles in cancer, aging and a number of human diseases. Due to its cellular scarcity and complex RNP assembly, the composition of human telomerase is still a matter of debate and available structural information on human telomerase is limited to a 30Å negative stain reconstruction. Here we present biochemical and cryo-EM studies on the human telomerase holoenzyme, revealing the first detailed architecture of an active telomerase RNP from a multicellular organism and new insight into its assembly and function. Our findings provide a structural framework for understanding human telomerase regulation and represent an important step towards telomerase-related clinical therapeutics.

References:
Blackburn, E. H. & Collins, K. Telomerase: an RNP enzyme synthesizes DNA. Cold Spring Harb. Perspect. Biol. 3, a003558 (2011).
Sauerwald, A. et al. Structure of active dimeric human telomerase. Nat. Struct. Mol. Biol. 20, 454-461 (2013).

Clemens Plaschka
Research Institute of Molecular Pathology

STRUCTURAL STUDIES OF NUCLEAR PRE-MRNA SPLICING

Splicing is an important step in gene expression, leading to the excision of non-coding introns from pre-mRNA. This process is mediated by the spliceosome, a large and dynamic machine comprising five snRNPs (U1, U2, U4, U5, U6) and several non-snRNP factors. Splicing begins with the recognition of conserved sequences at the intron 5' splice site and the internal branch point sequence, forming the U1–U2 snRNP prespliceosome. The prespliceosome then associates with the U4/U6.U5 tri-snRNP to assemble the pre-catalytic spliceosome. In my talk I will present the cryo-electron microscopy structures of the yeast Saccharomyces cerevisiae spliceosome in prespliceosome and pre-catalytic states at near-atomic resolution. The results reveal an induced stabilization of the 5' splice site during prespliceosome assembly, and provide insight into the roles of the human alternative splicing factors TIA-1 and LUC7-like in splice site selection. The results further suggest a mechanism for 5' splice site transfer to the U6 ACAGAGA region within the assembled spliceosome, for its conversion to the pre-catalytic spliceosome, and its subsequent activation to form the catalytic site. Taken together, the data provide a working model to investigate the early steps of splicing.

John L. Rubinstein
Canada Research Chair in Electron Cryomicroscopy
Senior Scientist, The Hospital for Sick Children
Professor, Departments of Biochemistry and Medical Biophysics, The University of Toronto

PROTON PUMPING V-TYPE ATPASES

Electrochemical proton gradients across energized membranes play a central role in numerous cellular processes. These gradients are established by large membrane-embedded protein complexes. The eukaryotic V-type ATPases are rotary enzymes responsible for acidification of intracellular compartments including endosomes, lysosomes, the Golgi, and exocytic vesicles. In specialized cells and tissues, V-ATPases acidify the renal tubule lumen and synaptic vesicles, and help dissolve bone minerals for bone maintenance. The function of these enzymes is controlled by a reversible disassembly mechanism, as well as different subunit isoforms. We have isolated V-ATPases and examined them by electron cryomicroscopy (cryoEM) to understand their function and regulation. In order to facilitate this process, we have also developed new biochemical, specimen preparation, and computational approaches for cryoEM.

Sharon Grayer Wolf
Weizmann Institute of Science

CHEMICAL AND STRUCTURAL INSIGHTS WITH CRYOSTEM TOMOGRAPHY

Cryo-TEM tomography (CET) is providing unprecedented sub-nm views into cellular ultrastructure for unfixed, unstained specimens in as-close-to-native state as possible. Recent developments in direct electron detectors, phase plates, and focused-ion beam milling of vitrified lamella has resulted in rapid progress, and structure determination of macromolecular machines *in-situ* is now actively being pursued in a growing number of labs.

Image information in CET depends on phase contrast, which requires coherent signal from elastically scattered electrons. Therefore, the mean free path for inelastic scattering limits the possible thickness of vitrified biological specimens for CET. Scanning TEM (STEM) mode is an attractive alternative to access thicker regions of the cell. STEM images are formed by point-by-point line scanning of a focused electron beam, and the electrons scattered by the sample are measured incoherently by bright-field (BF) and/or angular dark field (ADF) detectors. The mean free path for elastic scattering is the natural thickness limit for STEM, so for specimens that contain light elements, such as vitrified unstained biological cells, this provides around a three-fold increase in possible sample thickness, albeit at lower resolution (~ low nm).

STEM imaging mode also provides another interesting difference to TEM; as opposed to the phase contrast used in CET, CSTET contrast is based on mass. STEM contrast is extremely sensitive to elemental atomic number, and sample mass/thickness. STEM mode combined with Energy Dispersive X-ray Spectroscopy (EDX) allows for probing chemical content inside the cell with high spatial resolution.

I will provide samples in the talk of applications for these methodologies, including the characterization of Ca-phosphate granules in mitochondria and identifying different mineral deposits in organelles of Amoeba.

Elizabeth Wright
University of Wisconsin–Madison

USING CRYO-ELECTRON TOMOGRAPHY TO STUDY HOST-PATHOGEN INTERACTIONS

The bacterial flagellum is a complex propeller and is composed of three major units: the motor (basal body), the hook, and the filament. As a whole, the components work together to drive the bacterium through its environment. A number of studies of bacterial species indicate that flagellar filaments are assembled from several flagellin proteins as opposed to a singular flagellin. Many of the flagellins are differentially regulated, redundant in molecular weight, and function. While there may be some redundancy, there appears to be evidence that a single flagellin is essential for flagellar filament synthesis, motility, and virulence. *Caulobacter crescentus* expresses six flagellin proteins, and all the flagellins are present along the length of the flagellar filament. In this discussion of our structural study of the *Caulobacter* flagellum, we will highlight how we sought to ascertain which flagellin was essential and determine the high-resolution structure of both the full-length flagellum and the flagellin protein. We used a combination of mutagenesis experiments, (cryo-)fluorescence microscopy, and cryo-electron microscopy (cryo-EM) to generate and identify the appropriate mutants for structure determination.

Peijun Zhang
University of Oxford/Diamond Light Source

IMAGING VIRUS ASSEMBLIES IN SITU WITH CRYO-EM

With cryo-electron microscopy (cryoEM), structures of purified proteins and protein complexes can be routinely determined to near-atomic resolution using single particle analysis (SPA) method. Structures of macromolecular assemblies that are intrinsically flexible and dynamic, and often function in higher-order assemblies that are difficult to purify, have recently been analyzed to near near-atomic resolution using cryo-electron tomography and sub-tomogram averaging and classification (cryoSTAC). The study of these complexes in situ using cryoSTAC, coupled with cryoFIB and correlative and integrative imaging, opens a new frontier in structural cell biology. I will present technology development in cryoFIB and cryoSTAC, and recent studies of the virus assembly process within native cells to demonstrate the power of cryoET and sub-tomogram averaging for in situ structure determination.

SELECTED SPEAKERS

Maxim Armstrong
Bioengineering Department, University of California, Berkeley

MICRO-SCALE FLUID BEHAVIOR DURING CRYO-EM SAMPLE BLOTTING

Blotting has been the standard technique for preparing aqueous samples for single-particle electron cryo-microscopy (cryo-EM) for over three decades. This technique removes excess solution from a TEM grid by pressing absorbent filter paper against the specimen prior to vitrification. However, this standard technique produces vitreous ice with inconsistent thickness from specimen to specimen and from region to region within the same specimen, the reasons for which are not understood. Here, high-speed interference-contrast microscopy is used to demonstrate that the irregular pattern of fibers in the filter paper imposes tortuous, highly variable boundaries during removal of excess liquid from a flat, hydrophilic surface. As a result, aqueous films of nonuniform thickness are formed while the filter paper is pressed against the substrate. This pattern of nonuniform liquid thickness changes only slightly after the filter paper is pulled away, and, as a result, the thickness still does not become completely uniform. We suggest that similar topological features are produced during the standard technique used to blot EM grids and that these manifest in nonuniform ice after vitrification. These observations suggest that alternative thinning techniques, which do not rely on direct contact between the filter paper and the grid, may result in more repeatable and uniform sample thicknesses.

Simone Cavadini
Friedrich Miescher Institute for Biomedical Research, Basel

DNA DAMAGE DETECTION IN NUCLEOSOMES INVOLVES DNA REGISTER SHIFTING

The DNA in each cell is constantly exposed to several environmental and endogenous threats. This leads to a variety of DNA mutations that occur spontaneously (i.e, replication errors), after exposure to reactive chemical intermediates (i.e., reactive metabolites), or following exposure to UV-light. The global genome repair (GGR) pathway surveils genomic DNA for UV-light induced pyrimidine dimers, and targets these for repair by the nucleotide excision repair (NER) pathway (Ref. 1). Pyrimidine dimers including cyclo-butane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone 6-4 photoproducts (6-4PP) are found throughout the genome, including chromatin regions. Access to DNA packaged in nucleosomes is critical for DNA repair. In humans, the UV-DDB complex (comprised of DDB1 and DDB2) detects ultraviolet light-induced pyrimidine dimers, yet it remains unknown how these lesions are recognised in chromatin, where the histones and the two DNA gyres assembled into nucleosomes restrict DNA access.

Using single particle cryo-electron microscopy we determined structures for UV-DDB bound to nucleosomes bearing 6-4PP (Fig. 1) or DNA damage mimics at different positions (Ref. 2). These structures in combination with *in vitro* biochemistry, reveal that UV-DDB binds UV-damaged nucleosomes at lesions located in the solvent-facing minor groove without affecting the overall nucleosome architecture. On the other hand, for buried lesions facing the histone core, UV-DDB shifts the predominant translational register of the nucleosome, moving the damage to an exposed position compatible with UV-DDB binding. Our results predict how UV-DDB detects lesions that are occluded in tightly positioned nucleosomes in chromatin, and details a non-enzymatic mechanism for high affinity DNA-binding proteins, including transcription factors, to access nucleosomal DNA.

References

1) Sugasawa, K., *Mutat. Res*. 685, 29–37 (2010).
2) Matsumoto S.*, **Cavadini S.***, Bunker R. D.*, Grand R. S., Potenza A., Rabl J., Yamamoto J., Schenk A. D., Schübeler D., Iwai S., Sugasawa K., Kurumizaka H., Thomä N. H., *Nature*, published online 29 May 2019 // *These authors contributed equally

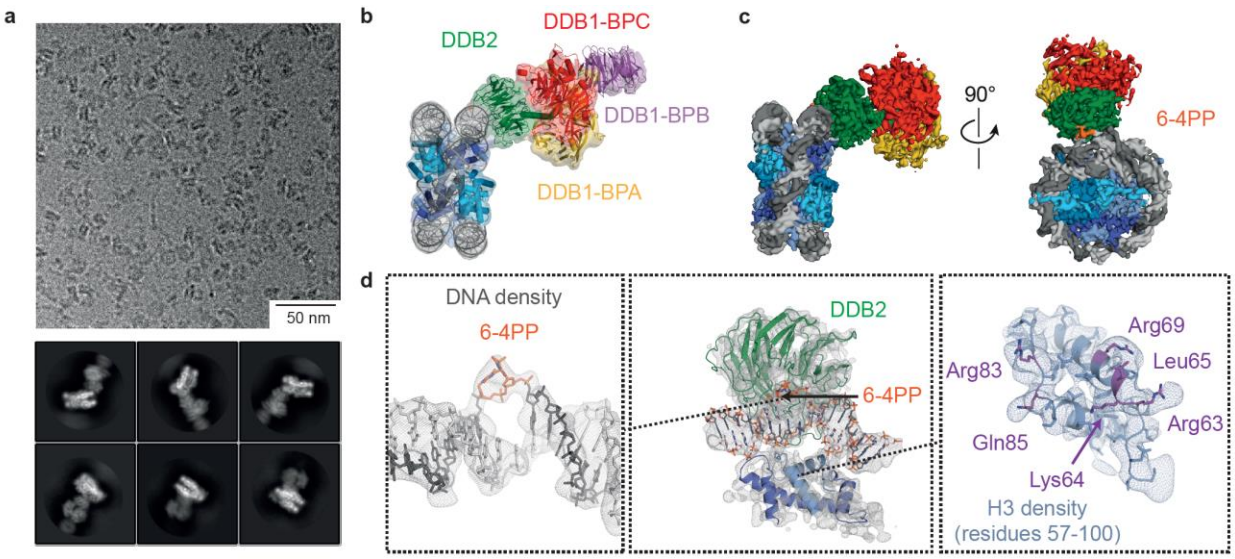

Fig .1 Cryo-EM structure of UV-DDB bound to a nucleosome carrying 6-4PP. a, Representative cryo-EM micrograph and reference-free 2D class averages. **b,** The NCP6–4PP–UV-DDB model fitted into the NCP6–4PP–UVDDB cryo-EM map. **c,** Orthogonal views of the NCP6–4PP–UV-DDB cryo-EM map refined with a mask that excluded the flexible DDB12-BPB domain. **d,** Representative, sharpened local-resolution filtered maps showing segments of the map shown in **c** for the damaged DNA duplex carrying 6-4PP and the DDB2–DNA–histone H3 interface.

Michele C. Darrow
TTP Labtech LTD.

CHAMELEON: NEXT GENERATION SAMPLE PREPARATION FOR CRYOEM BASED ON SPOTITON

In the workflow for high-resolution structure determination, improvements in microscope stability, direct detectors and image processing have shifted the bottleneck to sample preparation. The process of obtaining a film of vitreous ice of an appropriate thickness, with evenly distributed particles is not straightforward. Many of the current vitrification methods are highly variable, necessitating the costly step of screening each grid in an electron microscope (EM). Additionally, relatively large sample volumes are required and then lost during the process of blotting, and further grid losses are sustained during the manual handling required to transfer frozen grids into storage, and through poor traceability of storage locations.

The chameleon system is a blot-free, pico-litre dispense instrument for quickly and robustly freezing samples for use in cryo-EM. The chameleon system was developed from Spotiton [1,2] and uses self-wicking copper nanowire grids to form the thin sample film [3]. This process occurs 'on-the-fly' as the grid passes in front of the dispenser on its way to the cryogen bowl, resulting in a stripe of sample across the frozen grid.

This method of grid freezing provides many benefits:

• Blot-free high-speed plunging
• Automated grid handling
• Grid screening based on ice thickness
• Intuitive automated workflows
• Sample tracking and recording
• Cryogen feedback and control
• Potential impact on samples with air-water interface issues (preferred orientation, aggregation, denaturation)

In short, the chameleon is a sample vitrification system with walk-up usability that also creates opportunities for cutting-edge research despite poorly behaved cryoEM samples.

References:
[1] I Razinkov, et al. Journal of Structural Biology 195 (2016), p. 190-198
[2] V Dandey, et al. Journal of Structural Biology 202 (2018), p. 161-169
[3] H Wei, et al. Journal of Structural Biology 202 (2018), p. 170-174
[4] A Noble, et al. Nature Methods 15 (2018), p. 793-795

Benjamin Falcon
MRC Laboratory of Molecular Biology

CONFORMERS OF ASSEMBLED TAU IN HUMAN NEURODEGENERATIVE DISEASES

The ordered assembly of tau protein into abnormal filamentous inclusions in brain cells underlies many neurodegenerative diseases. We used cryo-EM to determine the high-resolution structures of tau filaments isolated from patient brain from a number of diseases, including Alzheimer's disease, Pick's disease and chronic traumatic encephalopathy. In parallel, we assessed antibody binding to intact filaments by immuno-EM to compare structured sequences within filaments across multiple disease cases and brain regions. Our results establish the existence of disease-specific conformers of assembled tau. Conformers are formed from conserved secondary structure motifs with markedly different conformations at turn residues. In addition, the identification of non-proteinaceous components within the ordered cores of tau filaments suggests a novel role for cofactors in assembly and conformer formation. These results pave the way to understanding the role of conformers of assembled proteins in neurodegenerative disease and may enable the rational design of tracer compounds and of inhibitors of assembly.

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René Henderikx
CryoSol World

VITROJET: BRIDGING THE GAP IN SAMPLE PREP

Over the past decade, cryo-electron microscopy (EM) became a powerful mainstream method in structural biology. With the increasing demand, reproducible sample preparation emerged as one of the main bottlenecks. We developed the VitroJet, that introduces several controllable techniques and combines them into one device with minimal operator intervention[1].

The device is supplied with a cassette of pre-mounted autogrids which are plasma cleaned inside the machine to ensure reproducible wettability. The autogrid then moves to the deposition chamber where it is maintained at its dewpoint temperature to prevent evaporation. A solid pin approaches the autogrid until the sample forms a capillary bridge between them. Translation of the pin results in deposition of an instantaneous thin film that does not need additional thinning, e.g. by blotting, nanowires or evaporation. The quality of the deposited layer is assessed with a camera for initial evaluation. After deposition, two simultaneous jets of liquid ethane vitrify the autogrid, targeting the center first. This results in higher cooling rates and allows for vitrification of autogrids, removing the need to mount grids under cryogenic conditions. Finally, the autogrid is stored ready to be loaded in the microscope.

The VitroJet provides higher level of control in sample preparation, where grid quality can be evaluated without being bound to the microscope. The automated technique enables users to optimize sample conditions faster in the race to obtain new and exciting structures. New developments and updates on the VitroJet will be presented.

[1] "Automated cryo-EM sample preparation by pin-printing and jet vitrification" *BioRxiv, https://doi.org/10.1101/651208*

Andrii Iudin
European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Cambridge, UK

EMPIAR: RECENT DEVELOPMENTS AND OUTLOOK

EMPIAR [1] is a public resource for raw electron microscopy images, and has been used for validation, methods development, teaching, training and community challenges. From the onset, EMPIAR has accepted image data from all imaging modalities covered by EMDB (single-particle, tomography, etc.). However, more recently we have also begun to allow archiving of data that cannot be stored in EMDB, such as 3D datasets from SBF-SEM, FIB-SEM and soft X-ray tomography (SXT) experiments, as well as 2D images used in integrative/hybrid modelling.

EMPIAR now has over 222 entries (~145 Tb), sees over 60Tb downloaded on average per month and has been cited in over 120 publications. In order to deal with yet larger volumes of data we are transitioning our back-end store to allow scalability on the Petabyte level. To facilitate deposition, we have developed pipeline software to automate submissions from microscopy centres. EMBL-EBI has recently developed the BioImage Archive which provides archiving for multiple imaging modalities and together with EMPIAR, for correlative modalities such as CLEM and CLXM.

In addition to archiving we are developing resources to facilitate the reuse and integration of EMPIAR data. The Volume Browser is a web-based, integrated viewer for structural data on scales from atoms to cells that makes structural information easily accessible to non-specialist users and thus facilitates knowledge discovery [2]. EMDB-SFF Toolkit (sfftk) and the Segmentation-Annotation Toolkit allow for semantic segmentation of 3D imaging data [3].

We would like community input on: a) Should deposition of raw data for certain modalities become mandatory? b) How long do we need to allow data to be held without release to promote the uptake of the pipelining architecture for automated deposition? C) Currently the main usage of EMPIAR involves the download of individual datasets. What are the use cases for analytics across data sets and would these benefit from having cloud computing resources close to EMPIAR data?

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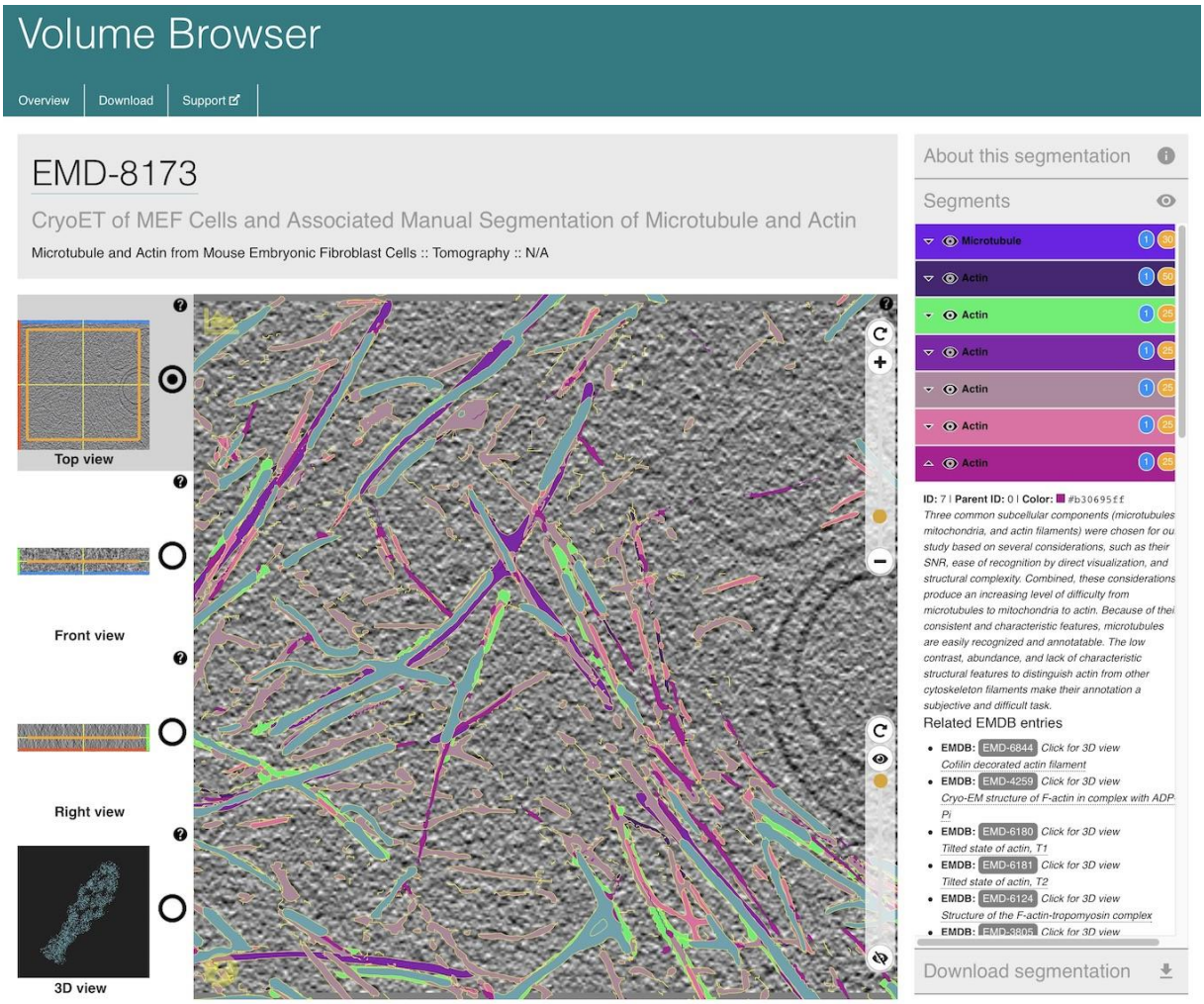


Prototype version of the Volume Browser.

Ahmad Jomaa
Department of Biology, Institute of Molecular Biology and Biophysics, ETH Zurich

CRYO-EM REVEALS THE MECHANISM OF SECA-MEDIATED CO-TRANSLATIONAL TARGETING OF MEMBRANE PROTEINS IN BACTERIA

Co-translational protein targeting to the cell membrane in bacteria or to the endoplasmic reticulum in eukaryotes is a universally conserved process to ensure the proper localization and folding of nascent membrane proteins. We recently solved the cryo-EM structure of the mammalian signal recognition particle (SRP) with its receptor in complex with the translating ribosome, which provided eukaryotic specific insights on the handover of the nascent protein to the translocation machinery on the membrane. Besides the well-studied SRP pathway, diverse pathways have been described that mediate this process, including the SRP-independent targeting pathway in eukaryotic cells and a SecA-mediated co-translational pathway in bacteria. The finding that SecA, previously known to mediate post-translational pre-protein translocation in bacteria, can also bind ribosome and mediate co-translational membrane protein targeting was a surprise, and currently there is no mechanistic description of this pathway. To better understand the mechanism of co-translational SecA targeting we determined the cryo-EM structure of SecA bound to the translating ribosome. Our very recent structure and biochemical data reveals that the transmembrane domain on the nascent protein is recognized by a composite binding pocket shared by both SecA and the ribosomal protein uL23 which explain the structural basis for the specificity of co-translational SecA recognition. Notably, the recognition of the nascent protein by the ribosome-bound SecA observed in our cryo-EM structure is distinct from the previously identified pre-protein binding sites of SecA in its post-translational targeting mode, which provides evidence for a dual mode targeting that is dependent on the nascent protein being targeted. In addition, our work demonstrates for the first time an active role of the ribosome in providing a timely shield for the emerging transmembrane domain on the nascent protein, in contrast to other pathways in which the chaperones or targeting factors are solely responsible for binding the nascent protein during membrane protein biogenesis.

Alexander Mühleip
Department of Biochemistry and Biophysics, Stockholm University

THE STRUCTURAL BASIS OF CRISTAE SHAPING IN TOXOPLASMA MITOCHONDRIA

Mitochondrial ATP synthase plays a crucial role in the maintenance of membrane morphology and membrane potential in mitochondria. It is generally accepted that by forming dimers, it shapes mitochondrial cristae, which is essential for organelle function. The transmembrane subunit a was shown to be responsible for proton translocation and underlying the dimeric structure. The human parasite Toxoplasma gondii has unique club-shaped cristae morphology and a minimal subunit a. To understand how the ATP synthase shapes cristae in *T. gondii*, we characterized it through a combination of cryo-EM, subtomogram averaging and electron cryo-tomography of whole mitochondria. We show that the native arrangement of the *T. gondii* ATP synthase is a hexamer, which is unprecedented in any known ATP synthase. The hexamer is composed of three dimers with no involvement of subunit a in dimer formation. On the supramolecular level, multiple hexamers are arranged around the cristae apices, thereby imposing membrane curvature. The unique *T. gondii* ATP synthase structure is dictated by 17 phylum-specific subunits and numerous bound cardiolipins, which we report for the first time. Thus, our results show that *T. gondii* has evolved a highly divergent macromolecular ATP synthase arrangement that provides the structural basis for its unique cristae morphology.

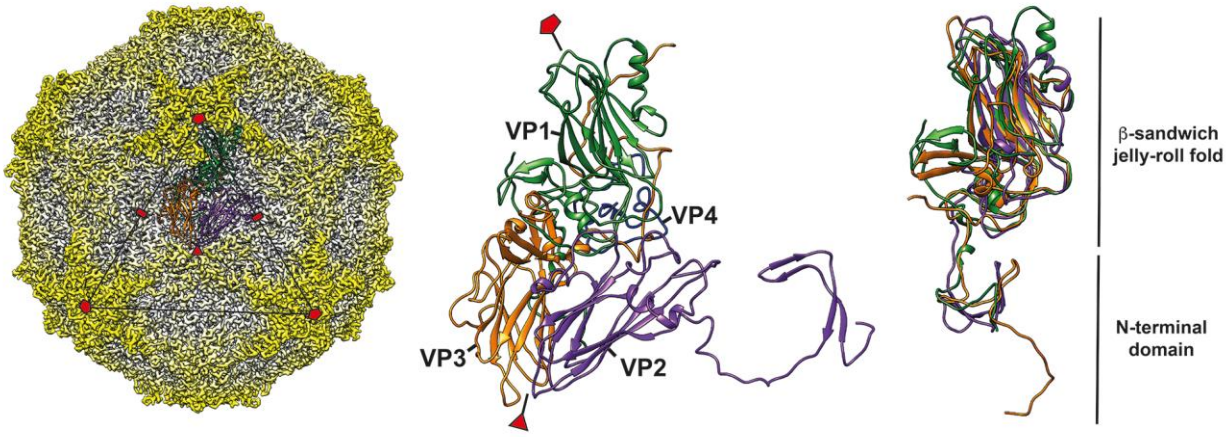
Anna Munke
The laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University

STRUCTURE OF A MARINE ALGAE VIRUS AND THE EVOLUTION OF PICORNAVIRIDAE VIRUSES

There is an uneven distribution of the major classes of RNA, DNA and retroid viruses among branches of host organisms. The RNA virome of marine metagenomic samples consists almost exclusively of the positive-sense single-stranded RNA ((+)ssRNA) viruses: the picornavirus-like superfamily. This implies that these viruses are an ancestral group from which the (+)ssRNA viruses of multicellular eukaryotes have evolved [Koonin, 2015].

The aim was to determine the structure of the diatom CtenRNAV-II virus and investigate the evolutionary relationship with members of the vertebrate *Picornaviridae* family as well as with other viruses of the *Picornavirales* order.

The structure of CtenRNAV-II was determined with single particle cryo-electron microscopy to a resolution (overall) of 3.1Å (FSC=0.143). This is the first high-resolution structure of an algae picornavirad. All four capsid proteins VP1, VP2, VP3 and VP4 were identified in the structure. The VP2 protein of CtenRNAV-II has a N-terminus domain swap, which is believed to be a primitive feature of the *Picoranviridae* viruses [Wang, 2015]. The domain swap as well as a structure-based phylogeny suggests that CtenRNAV-II belongs to an ancestral group of the *Picornaviridae* viruses. Additionally, VP1 exhibits a surface loop and does not follow the so-called canyon hypothesis that is proposed for many of the human pathogenic picornaviruses, suggesting that CtenRNAV-II has a unique way of recognising its host receptor.



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Adrianna Parizatto
Thermo Fisher Scientific

CRYO-EM ADOPTION – KRIOS G4

Cryo-EM possesses a long history that includes many groups of researchers who contributed to the evolution and revolution of cryo-EM in equally important ways. It has taken 40+ years of dedicated work for the structural biology community to develop the myriad technological advancements—detectors, automation, software and more—that have made modern cryo-EM possible.

Thermo Fisher Scientific pioneered this modern "Resolution Revolution" era of cryo-EM with its introduction of the Titan Krios™ transmission electron microscope in 2008. Recent technological advancements in the microscope design and imaging hardware, along with enhanced image processing and automation, have helped to catapult the technique's success. Many leading scientists have recently adopted the technique as one of the most critical tools in their laboratory. This increase in adoption, along with an unprecedented sharp rise in associated publications, led to Nature Methods naming cryo-EM as its Method of the Year for 2015 and 2017 Nobel Prize in Chemistry.

New generation of Krios (G4) launched in 2019 brings highest productivity, easy-of-use tools and new form factor which fits in a standard lab.

Pavel Plevka
Central European Institute of Technology, Masaryk University

STRUCTURE AND GENOME EJECTION MECHANISM OF STAPHYLOCOCCUS AUREUS PHAGE P68

Phages infecting *S. aureus* have the potential to be used as therapeutics against antibiotic resistant bacterial infections. However, there is limited information about the mechanism of genome delivery of phages that infect Gram-positive bacteria. Here we present the structures of *S. aureus* phage P68 in its native form, genome ejection intermediate, and empty particle. The P68 head contains seventy-two subunits of inner core protein, fifteen of which bind to and alter the structure of adjacent major capsid proteins and thus specify attachment sites for head fibers. Unlike in the previously studied phages, the head fibers of P68 enable its virion to position itself at the cell surface for genome delivery. The unique interaction of one end of P68 DNA with one of the twelve portal protein subunits is disrupted before the genome ejection. The inner core proteins are released together with the DNA and enable the translocation of phage genome across the bacterial membrane into the cytoplasm.

Urška Rovšnik
Department of Biochemistry and Biophysics, Stockholm University

CHARACTERIZING FUNCTIONAL STATES OF A MODEL LIGAND-GATED ION CHANNEL BY CRYO-ELECTRON MICROSCOPY (CRYO-EM)

Pentameric ligand-gated ion channels are important mediators of electrochemical signal transduction in the brain and other systems. Recent progress in protein biochemistry and structural biology has shed light on topology and possible gating mechanisms in these channels, yet the functionally relevant endpoints of activation and modulation remain unclear. In order to obtain alternative structural insights, we optimized protein purification, reconstitution, and grid preparation conditions for structure determination of a prokaryotic ion channel using cryo-electron microscopy (EM). We prepared samples at various presumed distributions of active and inactive states, with the goal of quantifying distinct classes within individual cryo-EM grids. Modification of carbon support, glow-discharging vapor, and blotting time were critical to improving particle distribution and orientation, and molecular simulations were harnessed to validate structural models. This work promises to inform methods development in cryo-EM of membrane proteins, and to populate the gating landscape in this important ion channel family.

Jana Skerlova
Department of Biochemistry and Biophysics, Stockholm University

CRYO-EM STRUCTURE OF THE MINIMAL PROGENITOR TOXIN COMPLEX OF BOTULINUM NEUROTOXIN X

Botulinum neurotoxins (BoNTs) are the most potent toxins known to man and are also used to treat an increasing number of medical disorders [1]. They target the neuromuscular junction and inhibit synaptic vesicle exocytosis in motor neurons, thereby causing paralysis. The molecular architecture of BoNTs comprises the receptor-binding domain, translocation domain, and zinc-protease domain. BoNTs are naturally co-expressed with a non-toxic non-hemagglutinin partner (NTNH) with which they form the minimal progenitor toxin complex to resist to low pH and proteases before they cross the intestinal barrier. The full-length structures of several BoNTs have been determined [2-5] and the structures of minimal progenitor toxin complexes of BoNT/A and BoNT/E are also available [6, 7].

We have recently identified and characterized a new botulinum neurotoxin serotype, BoNT/X. It shares the lowest sequence identity with other BoNTs and is not recognized by antisera against known BoNTs. BoNT/X cleaves its substrates at a novel site and is the only BoNT that also cleaves other non-canonical substrates [8, 9]. The only structural information currently available for this novel toxin is the structure of its zinc-protease domain [8].

We have determined the structure of the BoNT/X-NTNH complex using single-particle cryo-electron microscopy. This structure provides the molecular basis to understand the toxin's interactions with the protective partner and also the evolutionary relationships between BoNT serotypes. Moreover, it provides a platform to develop effective antidotes and to engineer new scientific tools and potentially also novel therapeutic toxins.

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Acknowledgements:
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POSTER PRESENTATIONS
DAY 1

Annnemarie Perez-Boerema
Department of Biochemistry and Biophysics, Stockholm University

Structural Diversity of Photosystem I

Many studies have shown how the basic building blocks of photosystem I (PSI) can differ between organisms, behaving in a modular fashion that allows different organisms to maximize the efficiency of sunlight driven energy conversion. In plants and algae PSI tends to be present as a monomer consisting of PsaA to L and PsaO with additional light harvesting complexes, whereas cyanobacteria PSI is often present as a trimer and, to a lesser extent as tetramer. In order to understand more of the structural basis causing the formation of tetramers over trimers, we solved a 3.2 Å structure of the PSI tetramer of *Anabaena* by cryo-EM and in order to understand more about organisms living in extreme conditions, we solved a 3.2 Å structure of a 'mini' PSI from *Dunaliella Salina*, a model organism to study acclimatization. The tetramer is composed of a dimer of dimers, thus showing very different contacts than those found in the trimer. This change is likely driven by changes in PsaL, and result in there being a significant gap between monomers, with interaction between the different monomers being mediated by key lipids. In *Dunaliella*, we found a 'mini' PSI, containing only subunits PsaA to F and PsaJ as well as Lhca1-4. The additional expected six subunits are missing (PsaG, PsaH, PsaI, PsaK, PsaL, PsaO). This 'mini' PSI could perhaps represent an acclimatization response to salinity conditions. Taken together, the structural information on PSI can paint a picture on how photosynthetic organism can adapt to their environment, whether it is by differences in subunits or in oligomeric states.

Björn Forsberg
Department of Biochemistry and Biophysics, Stockholm University

UNEXPECTED FEATURES AND STOICHIOMETRIC SYMMETRIES IN THE FUNGAL PYRUVATE DEHYDROGENASE COMPLEX

The pyruvate dehydrogenase complex (PDC) is a central component of all aerobic respiration, connecting glycolysis to mitochondrial oxidation of pyruvate. We determined a cryo-EM reconstruction of native and recombinant PDC particles from the filamentous fungus Neurospora crassa, showing how its X-component (PX) incorporates in fungi. We find that PX oligomerizes interior to the E2 core, producing a tetrahedral symmetry of the core complex which specifies the binding stoichiometry. The separate oligomerization and occlusion of PX reconciles variations previously found in PX-binding stoichiometry, suggesting a possible dynamic regulation mechanism. We also exemplify and discuss issues pertaining to reconstruction of complexes with multiple inherent symmetries, which imply broader implications regarding the interpretation of conventionally used classification methods.

Daniel S. D. Larsson
Uppsala University

STRUCTURE OF AN E. COLI 50S RIBOSOME ASSEMBLY INTERMEDIATE, THE SUBSTRATE OF METHYLTRANSFERASE RLMF

In all kingdoms of life, ribosomal RNA is posttranscriptionally modified. After processing of the nascent rRNA transcript, RNA folding, RNA modification and protein binding occurs in a coordinated manner, aided by additional ribosome assembly factors that facilitate the correct RNA folding and the appropriate RNA-protein interactions. In bacteria, the modifications are added by site-specific enzymes. There is limited structural knowledge of how these enzymes recognize their rRNA substrates that range from early rRNA states lacking proteins and other modifications to close-to-mature ribosomal subunits. RlmF is the methyl transferase responsible for the m^6A1618 modification of 23S RNA during ribosome biogenesis in *E. coli*. The modification site is only accessible at early stages of ribosome assembly, and in the mature 50S hides close to the ribosomal exit tunnel. We here set out to elucidate how RlmF recognizes its RNA substrate. Ribosomes were purified from an *rlmF* knockout strain and the 50S ribosomes were washed in high concentration of LiCl to form so-called LiCl core particles, an assembly intermediate analogue that lacks several ribosomal proteins. His-tagged recombinant RlmF was purified and *in vitro* shown to be active in methylation of LiCl core particles using tritiated S-adenosyl methionine. A ribosome-RlmF complex was purified by IMAC and sedimentation through a sucrose cushion. The protein content of the complex was confirmed by LC-MS/MS. A cryo-EM dataset for the RlmF-50S complex was collected at a Titan Krios microscope. The data is currently being analyzed to elucidate the structural mechanism of RNA modification. Interpretation of a 3.2 Å consensus reconstruction is on-going.

Dawid Zyla
ETH Zurich

THE ASSEMBLY PLATFORM FIMD IS ESSENTIAL FOR THE FORMATION OF THE MOST STABLE QUATERNARY STRUCTURE OF TYPE 1 PILUS RODS

Adhesive type 1 pili are long, supramolecular protein fibres anchored to the outer membrane of Gram-negative bacteria and are critical virulence factors of uropathogenic *E. coli* strains, as they mediate bacterial attachment to surface glycoproteins of urinary epithelium cells. The pilus rod is anchored to the outer *E. coli* membrane via the assembly platform FimD, which catalyses subunit assembly and translocation through the bacterial outer membrane. Besides FimD-catalyzed assembly of FimA *in vivo*, type 1 pilus rods also spontaneously assemble *in vitro* in a very slow reaction with a half-life of several days. We made the surprising discovery that pilus rods assembled *in vitro* show a dramatically diminished kinetic stability against dissociation and unfolding by the denaturant guanidinium chloride than pilus rods isolated from type 1 piliated *E. coli* cells. FimA assembly *in vitro* in the presence of the catalyst FimD, however, restored the higher stability of pilus rods assembled *in vitro*. Using unfolding/dissociation experiments in the presence of guanidinium chloride, we have discovered that the kinetic stability of the pilus rod depends on the method of its assembly. Pili assembled in vitro, in the absence of FimD, fold under kinetic control and reach a less stable conformation.

On the contrary, pili assembled *in vivo* or in a FimD-catalysed in vitro reaction, are approximately 1000-fold more stable (vs *in vitro*) and their folding is thermodynamically controlled. These results indicate that FimD is required for the assembly of the most stable type 1 pilus rod conformation. To investigate whether the stability difference can be explained by rearrangements in the quaternary structure of the rod we have determined several sub 3 Å resolution cryo-EM structures of pilus rods produced via the different assembly pathways. We also used ssNMR to pinpoint structural differences which could influence pilus stabilities.

Irina Iakovleva
Department of Chemistry, Umeå University

UNCOVERING THE AMYLOID FIBRILS STRUCTURE OF TRANSTHYRETIN USING CRYO-EM

Amyloidosis is a group of clinical disorders caused by the aggregation of specific proteins into abnormal extracellular deposits. Today, 31 different proteins have been linked to amyloid diseases including transthyretin-related amyloidosis (ATTR). ATTR affects about 50,000 people worldwide. The disease is progressive and lethal within 5 to 15 years after onset. ATTR occurs through the aggregation of either wild-type plasma protein transthyretin (TTR) or a mutated form. TTR is a homotetramer that under normal circumstances functions as a carrier of thyroxine and retinol binding protein. Until the 1990s, ATTR was considered as incurable disease, and today the only approved treatment is liver transplantation. In the last two decades, several new therapies have been proposed, including kinetic stabilization of TTR by small ligands such as tafamidis and diflunisal. According to previous studies, TTR aggregation cascade requires dissociation of the tetramer into monomers, which is the rate-limiting step and once the amyloidogenic monomer is formed, the process of downhill polymerization leads to rapid amyloid aggregation. However, the mechanism of TTR amyloid formation remains unclear due to the heterogeneity of the molecules involved and to the complexity of the aggregation steps. High-resolution structure of TTR fibril is important for understanding its assembly and pathological mechanism. The goal of this study is to determine a fibril structure of full-length TTR prepared under physiological conditions by cryo-electron microscopy.

Jianguo Zhang
Department of Medical Biochemistry and Biophysics, Umeå University

OBSERVATION OF TBE VIRUS REPLICATION IN MOUSE BRAIN BY CRYO-ELECTRON TOMOGRAPHY

Tick-borne encephalitis virus (TBEV) is a single strand ed positive sense RNA virus belonging to the family Flaviviridae. During its infection it specifically targets the brain and it is associated with serious, long-term neurological illness in humans During its replication, TBEV rearranges the endoplasmic reticulum (ER) membrane architecture to form a vesicle structure which provides a protected environment for viral RNA replication. How viral proteins stabilize this peculiar membrane shape is currently not known. Combining high pressure freezing off fresh, infected tissue with FIB milling and cryo-electron tomography, we were able to observe replication of the low-pathogenic TBEV relative Langat virus (LGTV) in specific regions of the mouse brain. We can clearly see TBEV replication vesicles in the ER membrane in the cryo-electron tomograms of infected brain and we will apply template matching and subtomogram averaging methods to study the associated protein densities. FIB-SEM volume imaging of resin-embedded infected tissue, as well as whole organ optical fluorescence tomography, are performed to add tissue and organ-level information on the infection.

Jingjing Zhao
Department of Materials and Environmental Chemistry, Stockholm University

A SIMPLE PRESSURE-ASSISTED METHOD FOR CRYO-EM SPECIMEN PREPARATION

Cryo-electron microscopy (cryo-EM) has made great impacts on structural biology. However, specimen preparation remains a major bottleneck. Here, we report a simple method for preparing cryo-EM specimens, named Preassis, in which the excess liquid is removed by introducing a pressure gradient through the EM grid. We show the unique advantages of Preassis in handling samples with low concentrations of protein single particles and micro-crystals in a wide range of buffer conditions.

Jiří Černý
CEIT Brno

BETTER STRUCTURES OF NUCLEIC ACIDS WITH DNATCO.ORG

We present a universal nucleic acids structural alphabet suitable for conformational analysis of DNA as well as RNA structures. The underlying local conformational classes of dinucleotide step (NtC) are characterized by seven torsion angles of the sugar-phosphate backbone, two torsions around glycosidic bonds, and three parameters describing the mutual orientation of bases within the step. Our previous definition of 44 DNA classes [1,2] was extended to incorporate also RNA conformers. We have obtained 84 conformational classes covering the combined structural variability of DNA and RNA.

The newly determined set of conformers substantially extends the previously determined set of RNA conformers [3]. All the classes are defined by means of hierarchical clustering of data from a sequentially non-redundant set of high-resolution crystal structures containing about 60 and 60 thousands steps for DNA and RNA, respectively. We have found that many of the previously defined conformational classes determined as specifically DNA [1] or RNA [3] are shared among nucleic acids. The assignment of nucleic acids conformation is available at https://dnatco.org as a freely accessible web service [4] for annotation and validation of nucleic acid structures.

We have implemented the conformational classes into the MMB program of Samuel Flores allowing conformational sampling and building of nucleic acid structures into experimental electron density. Further, the NtC based geometry restraints are generated for structure refinement in the Phenix suite of programs. A web based tool simplifying the model building and refinement is under development.

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Jose Miguel de la Rosa Trevin
Stockholm University

PUSHING THE AUTOMATION LIMITS OF CRYOEM DATA PROCESSING WITH SCIPION

Software development has been one of the keystones in the recent Cryo-EM revolution. New methods and faster implementations have made possible to compute in normal desktops or address more complex problems. Nonetheless, demands on the software side are increasing together with the needs of automation and more inexperienced users coming to the field. I will present some of the developments made in the Stockholm Cryo-EM facility to achieve more automation and get quicker feedback about the acquired data quality. By extending the functionality of the Scipion software framework, we have been able to routinely go to 2D classification and in many cases initial volume and 3D refinement. The new tools are also developed with extensibility in mind, where new algorithms can be quickly incorporated in the processing pipeline.

Joseph A. Lyons
Aarhus University

STRUCTURAL INSIGHTS INTO THE FUNCTION AND AUTO-REGULATION OF LIPID FLIPPASES

Department of Molecular Biology and Genetics, Aarhus University, Denmark Biological membranes are composed of bilayers, typically with highly asymmetric distributions of lipids that are of key importance for biomembrane function and dynamics, such as in secretory pathways, lipid signaling, cell-cell interactions, and motility. The asymmetric distributions are actively maintained by so-called lipid flippases and floppases that also counteract the passive scrambling of lipid gradients. Dominating contributions in eukaryotes come from lipid flippases of the P4 subfamily of P-type ATPases, where the majority of lipid flippases form a binary complex with a member of the Cdc50 family. These transporters are involved in the inward active translocation, hence “flipping”, of phospholipids across the membrane bilayer. Central questions on P4 ATPase lipid flippases address i) their overall architecture and function in the take up and flipping of lipids in membranes, and ii) how they are able to accommodate and transport a much larger substrate than other P-type ATPases, which usually transport small cations. Additionally, some P4-ATPases are subjects to auto-inhibition mediated through extended cytosolic termini, a feature that is shared with other P-type ATPases, like the plasma membrane calcium ATPase.

Here we present the first structures of a yeast lipid flippase, the phosphatidylserine (PS) specific flippase Drs2p/Cdc50p, with and without the auto-regulatory C-terminus, thus sampling inhibited and uninhibited conformations. Comparison of an autoinhibited and a truncated, PI4P activated state show re-arrangements of the cytosolic domains which transmit into flexible changes in the transmembrane domain. From these observations we propose a lipid substrate transport pathway and describe using unpublished structures of the mammalian lipid flippase ATP8a2/CDC50A how PS is translocated. Our results provide a structural framework to understand lipid translocation between membrane leaflets and the auto-regulation of lipid flippases.

Josephine D. Nissen
Aarhus University

INVESTIGATING THE ULTRASTRUCTURE OF THE AXON INITIAL SEGMENT BY CRYO-ELECTRON TOMOGRAPHY

The axon initial segment (AIS) is a functionally specialized region of the axon, which separates the somatodendritic domain from the axonal domain. It functions as the site of synaptic input integration and action potential initiation in neurons.

This project focuses on investigating structures of the AIS, in order to get a closer understanding of how dendritic signals are integrated and processed to give rise to a very controlled output; the action potential.

Super-resolution fluorescence microscopy has revealed a periodic structure of cytoskeletal and anchoring proteins with a spacing of approx. 190 nm in axons of cultured neurons (Xu et al, 2012 and Letterier et al, 2015). This well-ordered structure of proteins is likely having an impact on how action potentials are initiated and regulated in the AIS, and we wish to investigate how macromolecular complexes of membrane proteins such as ion channels, transporters and pumps are localized and functioning in relation to the periodicity of the cytoskeleton.

The end goal of the project is to gain biophysical insight into the higher-order 3D networks of membrane protein complexes in the AIS by cryo-electron tomography (cryo-ET) on FIB-milled lamellar sections of neurons. Our current approach involves antibody labeling and cryo-ET studies of cultured primary rat hippocampal neurons to obtain a low-resolution proof-of-concept model of periodical structures of the AIS.

Linda Sandblad
Umeå Core facility for Electron Microscopy, Umeå University

FOCUSED ION BEAM METHODS FOR ELECTRON TOMOGRAPHY

Recent development of effective workflow for cryo focused ion beam (cryo-FIB) and cryo-electron tomography has shown great importance for visualization of tissues, cells, subcellular structures and organelles at near native state. At the Core facility for Electron Microscopy, at Umeå University, we prepare electron-transparent lamellas from vitrified cells by cryo-FIB milling. Lamellas are subsequently analyzed by transmission electron microscopy (TEM), tomography and 3D image reconstruction using the IMOD software package, yielding low contrast but high-resolution information. Alternatively, we also use FIB and scanning electron micrscopy (FIB-SEM) for 3D cellular imaging by serial sectioning of resin-embedded samples, thus yielding a large reconstructed volume with beautiful intracellular contrast. In this study, we observe the morphology and cellular ultrastructure of fission yeast (*Saccharomyces pombe*). *Saccharomyces pombe* is an important model organism, popular for studying basic biology of eukaryotic cells. Both FIB techniques show different views of the yeast cells. The cryo-lamella and cryo-electron tomography method gives high resolution information of organelles and molecular complexes but limited information of spatial organization due to thin lamella (200 nm). In contrast, FIB-SEM volume imaging of resin-embedded yeast renders more spatial organization of the organelles and the full cell volume. Combining both techniques enables us to acquire a more complete view and to study eukaryotic cells at full perspective.

Jeongyoon Choi
University of Oxford, Biochemistry, Oxford, United Kingdom

TOWARDS UNRAVELLING TERTIARY STRUCTURE OF A MAMMALIAN LONG NON-CODING RNPs

Long non-coding (lnc) RNAs play crucial roles in many biological processes in the cell. Recent studies have suggested that many lncRNAs form ribonucleoprotein particles (RNPs), complex of RNAs and proteins, to function in the cell. One key example is Xist lncRNA, a master regulator of X chromosome inactivation in female mammals. Recent genetic and proteomic screening studies have provided a list of Xist RNA binding proteins (Xist RBPs). Cells depleting key Xist RBPs show reduced activity in X chromosome silencing. Secondary structural analysis of partial Xist RNAs have been performed by several research groups. However, we still lack understanding of tertiary structure of native Xist RNP. Here, we will discuss our efforts and progress in unravelling tertiary structure of the Xist RNP.

Alexander Jussupow
Technical University of Munich (TUM)

THE IMPACT OF CARDIOLIPIN ON THE STRUCTURE, DYNAMICS AND ACTIVITY OF COMPLEX I

Complex I is an energy-converting membrane protein in aerobic respiratory chains that reduces quinones and pumps protons across the membrane. Cardiolipin plays an important role for the activity of complex I, but despite recently resolved cryo-EM structures of complex I, the exact function of cardiolipin still remains unclear. Here we show using a combination of millisecond coarse-grained, atomistic molecular dynamics simulations, and cryoEM data how cardiolipin induces global conformational changes that affects the quinone dynamics in complex I. We also show that protein-lipid interactions increases the likelihood of cardiolipin diffusion into the surroundings of complex I, with possible implication for the supercomplex formation. The findings provide insight into how biological membranes may modulate the structure and activity of proteins.

Sigrid Noreng
Oregon Health and Science University

STRUCTURE OF THE EPITHELIAL SODIUM CHANNEL BY CRYO-EM

Epithelial sodium channel (ENaC) is the rate-limiting step of sodium reabsorption, regulating extracellular fluid volume, and thereby blood pressure. The importance of ENaC in sodium homeostasis is emphasized by gain of function mutations causing severe hypertension, as in Liddle's syndrome, and in loss of function mutations causing the neonatal salt-waste disorder Pseudohypoaldosteronism type 1. ENaC assembles as a heterotrimer, consisting of three homologous subunits, alpha-beta-gamma, and belongs to the superfamily of ENaC/degenerin. Members of this protein family are voltage-independent, sodium selective and amiloride-sensitive. Currently, the acid sensing ion channel (ASIC) is the only member of the ENaC/degenerin family with a known structure. A series of published structures revealing different conformational states of ASIC have elucidated stoichiometry and gating mechanism for this ion channel. While this work provided insight into properties shared among the proteins in the same family, there are significant characteristics unique to ENaC not found in ASIC. One such property includes the unusual gating mechanism of ENaC where the alpha and gamma subunits are cleaved at distinct extracellular sites, causing release of the inhibitory domains to open the channel. Another distinct property of ENaC includes a higher selectivity for Na^+ over K^+ at 100:1 compared to ASICs, which varies between 30:1 and 3:1. Here we present the structure of human ENaC in the uncleaved, resting state determined by single-particle cryo-electron microscopy. The structure reveals that ENaC assembles with a 1:1:1 stoichiometry of alpha-beta-gamma subunits arranged in a counter-clockwise manner. Each subunit of ENaC resembles a hand similar to the analogy of ASIC subunits. The shape of each subunit is reminiscent of a hand with key gating domains of a ‘finger’ and a ‘thumb.’ Wedged between these domains is the elusive protease-sensitive inhibitory domain poised to regulate conformational changes of the ‘finger’ and ‘thumb’; thus, the structure provides the first view of the architecture of inhibition of ENaC.

Edit Szodorai
Department of Medical Biochemistry and Biophysics, Karolinska Institutet

SECRETAGOGIN INTERACTOME CONVEYS CALCIUM SIGNALING OF INSULIN RELEASE, B-CELL IDENTITY AND SURVIVAL

Calcium-binding proteins (CBPs) are essential mediators of intracellular calcium (Ca^{2+}) concentration-dependent cell signalling. Extracellular stimuli driven transient Ca^{2+} spikes allow precise modulation of CBPs, so called Ca^{2+} sensors. Upon binding, they undergo conformational changes enabling formation of protein-protein interactions which assure rapid, dynamic decoding of short-lived intracellular Ca^{2+} signals.

Secretagogin (SCGN), a hexa EF-hand Ca^{2+} sensor, has been shown to be predominantly expressed in the central nervous system and in endocrine glands playing crucial role in hormone and neuropeptide secretion. It is one of the most abundant proteins of endocrine pancreatic β-cells and suggested to regulate insulin release via interaction with cytoskeletal proteins and components of vesicle-mediated trafficking. Accordingly, SCGN loss-of-function correlates with decreased insulin secretion and leads to diabetic phenotype.

Ca^{2+}-dependent SCGN interaction network in pancreas was investigated to provide a more comprehensive picture on SCGN-mediated signal transduction. In INS-1E cells the analysis revealed 13 proteins involved in protein folding including members of the chaperonin containing T complex. Moreover, we detected enzymes of deubiquitination such as ubiquitin carboxyl terminal hydrolase USP9X and USP7. These findings pinpoint abnormal protein folding and degradation with subsequent β-cell loss that may explain impaired insulin secretion in SCGN knock downs (1). In a second study, we described SCGN interacting proteins in mouse embryonic pancreas and identified subunits of the 26S proteasome complex. Ca^{2+}-dependent interaction of SCGN with the 26S proteasome modulates proteasome activity which determines availability of transcription factors defining β-cell identity (2).

Taken together, our results indicate SCGN to be a pivotal molecular hub in many fundamental cellular processes conveying Ca^{2+} signals in the endocrine pancreas. Furthermore, we address that detailed one on one analysis of putative partner proteins has the potential to depict molecular background and functional consequences of these interactions.

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Andrea Di Luca
Center for Integrated Protein Science Munich at the Department of Chemistry, Technical University of Munich

GLOBAL MOTIONS AND LOOP REGIONS ARE INVOLVED IN THE DEACTIVATION OF COMPLEX I

Complex I (NADH:ubiquinone oxidoreductase) is a redox-driven proton pump that serves as a primary electron entry point in aerobic respiratory chains. In absence of substrate, complex I undergoes in some species an active-to-deactive (A/D) transition, entering a deactive state (D) that shows a low turnover rate (1). Recent Cryo-EM structures of the complex in both A and D states shed light on some of the conformational changes involved in this transition (2, 3). By employing Elastic Network Models (ENM), we highlight how these structural changes are related to low-frequency collective motions of the enzyme (4). We identify high-strain regions of the enzyme during the transition and relate these to the elements that may transduce redox energy into the proton pumping machinery.

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MOLECULAR MECHANISM OF ANTIBIOTIC RESISTANCE MEDIATED BY ABCF ATPASES

Translation is one of main processes in all living organisms, therefore it is not surprising that the majority of known antibiotics target the ribosome – the machinery of translation. Translation on ribosome is a precise process of deciphering information from mRNA to protein. In order to respond to different growth phases, stress conditions and environment changes, the process of translation is assisted by numerous translation factors. Among them are recently characterized ABCF translational factors, which for decades were believed to be part of unidentified ABC transporters. Antibiotic resistant (ARE) ABCFs were found in clinical isolates where they confer resistance to a broad spectrum of antibiotic classes: oxazolidinones, macrolides, lincosamides, pleuromutilins and streptogramins, phenicols and tetracyclines. As some of these antibiotics are considered to be last resort antibiotics for human use, we are motivated to uncover their molecular workings and shed some perspectives on counteracting them.

At the moment we are focused on characterization of distribution of ARE ABCFs, the connection between each protein class and its antibiotic resistance with a goal of making it possible to predict antibiotic resistance of newly identified ABCFs based on their sequences. I will present our data on evolution of ABCFs and mechanistic details of action of ARE ABCFs VgaA, LsaA and VlmR proteins resolved by biochemistry, microbiology and structural biology.

Lotta Happonen
Division of Infection Medicine, Lund University

STRUCTURAL BIOLOGY AND STRUCTURAL MASS SPECTROMETRY IN HOST-PATHOGEN PROTEIN-PROTEIN INTERACTIONS

Many significant bacterial pathogens produce evolutionary optimized proteins that form interactions with human host proteins to evade the immune defenses, to acquire metabolites and to facilitate adherence. A fundamental challenge in medical microbiology is to structurally characterize these protein-protein complexes arising at the interface between a pathogen and its host, with the potential of profoundly advancing our understanding of the molecular level detail of bacterial infections. One such important human pathogen is the Gram-positive *Streptococcus pyogenes* bacterium causing diverse clinical manifestations ranging from mild and common local infections to life-threating systemic diseases like sepsis, meningitis and necrotizing fasciitis. Currently, a considerable portion of the streptococcal proteins used in host immune evasion are poorly characterized.

Here, we use an integrative structural biology approach to characterize the complex formed between the G-related alpha-2-macroglobulin binding -protein (GRAB) from *S. pyogenes* and the broad-range human protease inhibitor alpha-2-macroglobulin (A2M). This interaction is known to protect the bacteria against proteases of the human immune system, but the mechanism of the interaction is unknown. Here, we demonstrate using nuclear magnetic resonance (NMR) spectrometry and hydrogen-deuterium exchange mass spectrometry (HDX-MS) that the streptococcal GRAB is an intrinsically disordered protein without a folded 3D structure when in solution. We further describe the interaction interface of GRAB and A2M using an integrated combination of single particle electron cryo-microscopy (cryoEM), HDX-MS and targeted cross-linking mass spectrometry (TX-MS). These results suggest that GRAB binds on the outside of A2M, possibly blocking the receptor-binding domain on A2M crucial for endocytic clearance of the complex.

The integrative methods presented here allows us to describe host-pathogen protein-protein interaction interfaces in high detail. The identification and characterization of such protein interaction interfaces has great medical importance, as perturbation of these interaction sites could lead to the discovery of new drugs and other treatment strategies to prevent infections.

Max E. Mühlbauer
Center for Integrated Protein Science Munich, Technical University of Munich

CHARGE-TRANSFER DYNAMICS IN THE MEMBRANE DOMAIN OF RESPIRATORY COMPLEX I

Respiratory complex I is the main entry point for electrons into respiratory chains making the intricate enzyme a key player in biological energy conversion. The free energy from transferring two electrons from NADH to ubiquinone in the enzyme's hydrophilic domain is used to power the transduction of four protons across biologic membranes in its membrane domain. These charge transfer processes are tightly coupled despite being separated by up to 200 Å.

Although a plethora of high-resolution structures has emerged recently, the mechanistic principles of this long-range signal transduction remain elusive. Here, we employ computational quantum chemical and classical molecular dynamics simulations to probe conserved key elements of the signal propagation and proton transduction processes. We observe a tight coupling between the proton transfer energetics, the hydration state of the membrane domain and the dynamics of conserved charged residues. The work provides a basis for understanding how the long-range signal transduction affects the proton pumping reaction in antiporter-like subunits of complex I.

POSTER PRESENTATIONS DAY 2

Marta Šiborová
Structural Virology, Central European Institute of Technology

STRUCTURE OF BACTERIOPHAGE SU10

Bacteriophage SU10 was isolated from sewage water from Käppala waste water treatment plant in Stockholm. It belongs to the family *Podoviridae* and infects wide range of *E. col*i strains. The phage has 77 kbp dsDNA genome, prolate capsid and short tail with base plate.

We determined the molecular structure of capsid, portal, tail-base plate complex of SU10. Capsid is formed by 11 pentons and 110 hexons of major capsid protein, which has HK97 fold. The dodecameric portal complex features a prolonged crownbarrel on the top, similar to that of phage P22. Phage particle is decorated by three types of fibers, all present in six copies. Collar fibers are connected to the neck protein complex. Long tail fibers are connected to the upper tail protein. Short tail fibers are positioned on bottom part of the base plate. Hydrolytic tail needle is pointing out from the center of the base plate.

Our high-resolution reconstruction of the phage SU10 is the first reported detailed insight into the structure and build-up of Gram-negative bacteria attacking phages.

Natalia de Val
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Team Lead, Cryo-EM, Center for Molecular Microscopy, Center for Cancer Research (CCR), National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, Maryland, USA

USING ELECTRON MICROSCOPY TO ENHANCE THE KNOWLEDGE OF BIOLOGICAL SYSTEMS

It is quite impressive to think about the amount of time that has passed before we could get to the point of obtaining 3D reconstructions below 3A resolution by single particle cryo-EM. Four decades after the first developments that laid the groundwork for single particle cryo-EM, a high-resolution reconstruction of haemoglobin in solution, determined using cryo-EM, was presented (*Nature Communications*, 8, 16099, 2017). However, in less than a decade, single particle cryo-EM, has evolved very fast. Recent technical developments as the quality of the TEM microscopes, the Volta phase plate, as well as the introduction of direct detector cameras were key to dramatically increase the signal-to-noise ratio and the spatial resolution. Automation of data collection using different software as Serial-EM, EPU or Leginon, has also significantly advanced the field.

Several examples of collaborations and work done at the Center for Molecular Microscopy (CMM) from HIV-1 Envelope in complex with broadly neutralizing antibodies to gp5-gp4 DNA complex will be presented to illustrate the power of the single particle analysis. The use of cryo-Electron Tomography as well as micro-Electron Diffraction is increasing in my group. Some other examples will be presented during this talk highlighting the advantages of using these techniques.

Pavol Bárdy
Faculty of Science, Masaryk University

STRUCTURE AND DNA DELIVERY MECHANISM OF RHODOBACTER CAPSULATUS GENE TRANSFER AGENT

Gene transfer agents (GTAs) are extracellular particles that enable high-frequency horizontal gene transfer among prokaryotes and thus accelerate their evolution [1]. GTAs are derived from phages that were independently acquired by several bacterial and archaeal lineages [2]. In spite of their importance for adaptation and diversification of prokaryotes, the structure and mechanism of DNA delivery of GTAs are unknown. Here we used cryo-electron microscopy to show that GTA of *Rhodobacter capsulatus* resembles bacteriophage from the family *Siphoviridae* with several unique features. The DNA-containing head of the GTA is shortened in the direction of GTA tail relative to the regular icosahedral heads of phages. This results in T3 compressed icosahedron with ~50% reduction of the DNA packaging capacity. It represents the smallest possible size of a capsid, which can be assembled in portal-containing viruses. Unlike in the previously studied phages, attachment of the GTA tail to the head is reinforced by the interaction of the neck adaptor protein with the capsid. Tape-measure protein, which determines the length of the GTA tail, exhibits similarities to proteins of phages with both short and long tails and may, therefore, correspond to their common ancestor. DNA-delivery is initiated by the attachment of GTA head-spikes and tail-fibers to the host capsule. Subsequently, the GTA baseplate interacts with the bacterial outer membrane, irreversibly disassembles, and releases the cell-wall hydrolase, which is located inside the baseplate. The tape-measure protein is ejected together with GTA DNA into the bacterial periplasm, from where DNA is transported into the cytoplasm by cellular transformation complex. Our results reveal novel DNA-delivery mechanism present in GTAs and related phages.

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Rasmus Kock Flygaard
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ATP SYNTHASE IN TOXOPLASMA SHAPES THE MITOCHONDRIA THROUGH PREVIOUSLY UNSEEN HIGH OLIGOMERIC STATES

F-type ATP synthase is ubiquitous enzyme found in all kingdoms of life. In mitochondria, ATP synthases dimers reside in the inner membrane. It is generally accepted that dimeric assemblies of the ATP synthase induce curvature of the membrane that is universally essential for mitochondrial function. Human parasites *Toxoplasma gondii* have unique mitochondrial morphology, and how it achieved is not known. To answer this question, we investigated *T. gondii* ATP synthase by a combination of single-particle cryo-EM and cryo-ET. Using high-resolution reconstruction of the dimeric ATP synthase, a near-complete *de novo* atomic model has been constructed revealing a unique F_o sub-complex with no resemblance to any reported structure of the ATP synthase. In addition, we discovered a hexameric arrangement, showing for the first time a cyclic supramolecular assembly of the ATP synthase. Formation of the ATP synthase hexamer is also facilitated by the highly divergent multisubunit membrane domain F_o and specifically bound lipids. Finally, using tomographic reconstructions we show that in the mitochondrial inner membrane ATP synthase hexamers assemble into dome-shaped complexes residing on the apices of the tear-drop shaped cristae. Taken together, these data explains how the unusual *T. gondii* mitochondria are shaped by the modulation of the ATP synthase.

Rei Matsuoka
Department of Biochemistry and Biophysics, Stockholm University

STRUCTURAL BASIS FOR NA+/H+ ANTI-TRANSPORTATION MECHANISM OF NHA2 IN SLC9B FAMILY

In mammals, 13 NHE isoforms are currently known; NHE1-13 (SLC9A1-13). NHA2, also known as SLC9B2, was only recently identified [1]. NHA2 correlates with the long-sought sodium/lithium countertransporter linked to the pathogenesis of diabetes mellitus and essential hypertension in humans [2]. As yet, there is no structural information for NHA2. Each monomer consists of a transporter domain (11-14 TMs, 460 aa) that carries out ion-exchange, and a dimer domain [3,4]. Crystal structures of several bacterial homologues have been determined. Interestingly, in the bacterial homologues, there is strong evidence the ion-transporting domains undergo large rearrangements to carry the ions across the membrane [3]. In this elevator-model, the dimerization domain is fixed and acts as a scaffold for the highly mobile transporting domains. Our goal is to capture multiple and functionally relevant conformational states of NHEs and NHA2 transporters by single-particle cryo-EM. This will enable us to establish and compare the alternating-access mechanism in the NHE vs. NHA2 proteins.

A total of 4620 micrographs were recorded and subjected to motion correction and dose-weighting of frames by MotionCor2 (Zheng et al., 2017). CTF parameters were estimated by applying CTFFIND4.13 (Rohou and Grigorieff, 2015). All micrographs were selected for image processing was performed using RELION3.0 (Zivanov et al., 2018). Approximately 1228 particles were manually picked to generate templates for automated particle selection. A first round of 2D classification included 1,462,831 particles. Using ab initio model from RELION was build using 2D classes from 190,558 particles. After making *ab initio* model, we used 3D classification to remove poor particles. After several rounds of 3D classification, we could obtain a good initial model for refinement. During the refinement, we optimized parameters and finally we could obtain around 4.9Å resolution map of NHA2.

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CRYO-EM OF SELF-ASSEMBLED FAK 2D CRYSTALS REVEALS DOMAIN REARRANGEMENTS AND OLIGOMERIZATION UPON MEMBRANE BINDING

Focal adhesion kinase (FAK) is a key component of focal adhesion sites, enabling mechanical sensing between the extracellular matrix (ECM) and actin microfilaments. This tyrosine kinase is involved in the regulation of cell migration, adhesion and survival processes. The phospholipid PIP_2 plays a special role with FAK by inducing preferred membrane binding orientations[1]. In the presence of a PIP2 monolayer, a FAK construct containing the kinase and FERM domains (74 kDa) oligomerizes and self-assembles into a 2D crystal form. These crystals are however poorly ordered, diffracting to no further than 25 Å. We then used the FOCUS package[2] to export cryo-EM data of FAK 2D crystals for processing with single-particle analysis software. The alignment and classification of local patches of the 2D crystals corrects for distortions and disentangles co-existing forms of crystal packing, respectively[3]. This approach resulted in 6 Å reconstructions of FAK with and without the AMP-PNP ligand. These maps reveal the domain organization of FAK bound to a lipid membrane after recruitment to focal adhesion sites, providing experimental insight into the intermediate steps between initial auto-inhibition[4] and subsequent activation by mechanical forces[5].

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CRYO-EM STRUCTURE OF HUMAN MITOCHONDRIAL LON PROTEASE

Human mitochondrial Lon (HuLonP) is the major mitochondrial matrix protease essential for mitochondrial function. Lon belongs to the AAA+-protease family and is a homo-hexamer that uses energy from ATP hydrolysis to recognize, bind and translocate its substrate into a proteolytic chamber where it hydrolyses the protein. Here, we present the structure of a full-length human mitochondrial Lon determined by single-particle cryo-EM to a resolution of 3.6 Å resolution. We find that in the absence of a protein substrate, the hexameric Lon arranges itself into an open helical conformation with an 8Å translational rise in the seam protomer.

The globular substrate recognizing N-terminal, LAN domains of oppositely placed protomers dimerize to form three flexible legs. Inside the chamber, the inter-protomer communication is not confined to the previously known Arg finger as reported by bacterial Lons. Motifs like ISS and flexible loops play equally important roles in protomer communication. Extensive interaction both inside and outside the chamber, put the substrate free human Lon under strict allosteric regulation. The structure reveals the first overall complete organization of the human Lon protease.

Samuel Flores
Department of Biochemistry and Biophysics, Stockholm University

FAST MULTISCALE METHODS EXTRACT MORE INFORMATION FROM MIXED-RESOLUTION DENSITY MAPS OF RIBONUCLEOPROTEIN COMPLEXES

Compared to crystallography, CryoEM offers a more robust treatment of large molecules in heterogeneous structural states. Modeling is challenging in this regime, as all-atoms simulation methods such as Molecular Dynamics (MD) struggle with large molecules, especially when modeling very large scale conformational changes. Even smaller macromolecules such as nucleic acid quadruplexes can be challenging since they be able to access many conformational states with similar energy and backbone structure. Multiscale methods, which treat different regions of the macromolecule at different levels of flexibility and physics, offer distinct advantages here, in particular when tapping recently published structural libraries such as the Cerny Lab's Nucleotide Conformers (NtCs). We have previously shown that even the translocation-completion step of the ribosome, which takes on the order of a tenth of a second in vitro, can be modelled on a laptop in under an hour. Now we show that for quadruplexes and other (relatively) small nucleic acid structures, we can stochastically try many possible conformational variations and quickly relax to the correct conformation, with results that are more accurate than the experimental resolution would directly imply. Thus we can solve the large scale conformation even in low-resolution regions, and simultaneously infer the detailed structure in regions of higher resolution.

Selma Dahmane
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IN SITU STRUCTURAL STUDIES OF ENTEROVIRUS REPLICATION USING FIB MILLING AND CRYO-ELECTRON TOMOGRAPHY

The aim of this study is to gain structural insights into the genome replication of human enteroviruses (EVs). EVs drastically reorganize the internal membranes of a cell within hours of infection, generating replication complexes (RCs), which are the sites of viral genome replication. Some molecular determinants of RC morphogenesis have been identified, and resin-embedding EM has indicated the drastic membrane remodeling involved in RC formation. However, such approaches fail to reveal the macromolecular structural organization of RCs in cells. To further our understanding of RC assembly and activity, we use cryo-electron tomography (cryo-ET) to determine 3D structures of enteroviral RCs in infected cells. The recently developed cryo-focused ion beam milling technology is used to make RCs inside infected cells accessible to structural studies using cryo-ET. The determined structures will reveal the supramolecular organization of viral proteins and RNA at EV RCs. The findings will clarify the long-standing question regarding the relation between membrane topology and the RNA synthesis machinery of the highly medically relevant EVs. Our preliminary data show a close proximity between the replication and assembly of the viruses, as well as membrane connectivity with autophagy organelles.

Søren Kirk Amstrup
Department of Molecular Biology and Genetics, Aarhus University

INVESTIGATION OF PHOSPHONATE DEGRADATION BY THE CARBON-PHOSPHORUS LYASE COMPLEX

When exposed to phosphate starvation some bacteria can use alternative sources of phosphate. One of these alternative sources are phosphonates, which are chemical compounds characterized by having a covalent bound between carbon and phosphate. Of the three known pathways for metabolizing phosphonates the C-P lyase pathway carries the widest substrate specificity and works by using radical chemistry to liberate phosphorous and eventually incorporate it into 5-phosphoribosyl-α-1-diphosphate (PRPP). The C-P lyase pathway is dependent on the *phn*-operon, which in E. coli contain 14 cistrons (*phnCDEFGHIJKLMNOP*), the first 4 cistrons code for an ABC-transporter and a transcriptional regulator whereas the last 10 code for the enzymatic proteins.

A biochemical pathway for the breakdown of phosphonates by the C-P lyase proteins have been proposed though it have yet to be structurally proven. A former member of our lab solved the structure of the PhnGHIJ complex using crystallography(1). This structure comprises a heterooctamer with C2 symmetry where the subunits share core folds in two pairs, PhnH-PhnJ and PhnG-PhnI. The PhnGHIJ structure raised questions towards the proposed biochemical pathway demanding large rearrangements of the structure. To pursue this flexibility, we have used EM techniques due to instability and flexibility of the complex which complicated crystallization.
We have managed to solve a single particle cryo-EM map of the PhnGHIJK complex solved to 3.8 Å resolution. This structure revealed that the PhnK subunit breaks the C2 symmetry of the PhnGHIJ core complex and contains more flexibility. Interestingly PhnK is an ATP-binding cassette (ABC) protein, these usually form dimers which can cleave ATP, this has not been observed yet for the PhnGHIJK complex. The next gene in the *phn*-operon *phnL* codes for another ABC, which could together with PhnK cleave ATP and break the rigid PhnGHIJ core complex.

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Tales Rocha de Moura
KTH Royal Institute of Technology

STRUCTURAL STUDIES OF THE PANNEXIN 1 MEMBRANE CHANNEL

Pannexins play an important role in various physiological and pathological events, and are involved in the control of oxygen delivery, control of ciliary beat in airway epithelia, pathogen sensing by immune cells, propagation of calcium waves in various tissues including astrocytes, and possibly in several sensory mechanisms. They are also involved in pathological settings including ischemia, inflammation, myocardial infarction and secondary cell death. ATP release is a common denominator in all of these functions of Panx1. However, structural information is still scarce limiting the understanding of its molecular mechanisms.

To move towards a better structural understanding and shed light on the mechanisms involved in its functions, the aim of this project is to determine the Panx1 channels structure using cryo electron microscopy (cryo-EM).

For the protein production a Sf9 expression system has been established using baculoviruses. The expressed protein was purified by affinity and size exclusion chromatographies, reaching high purity. The sample was used for grid preparation in two states, encased in a detergent micelle and reconstituted in protein lipid-nanodiscs. The corresponding grids were used for cryoEM experiments and the collected data analysed by current methods. The data collection from the protein encased in detergent micelle generated particles that allowed us to reconstruct pannexin to an intermediate resolution. However, we observed a high background which resulted in a low resolution structure. As a step towards higher resolution, we were successful in obtaining a homogeneous population from the protein reconstituted in nanodiscs, and were able to obtain classes showing domains similar to other pannexin related channels. The data showed particles with a preferred orientation but enable us to generate a preliminary reconstruction. The 3D reconstruction and the classification were calculated without imposing any symmetry and despite these limitations we can clearly observe secondary structure elements. As future perspective, the sample and data collection strategies are being optimized in order to increase the total amount of particles and circumvent the preferred orientation limitations, enabling us to reach higher resolutions.

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IN-SITU STUDIES OF ALPHAVIRUS REPLICATION COMPLEXES BY CRYO-ELECTRON TOMOGRAPHY

Alphaviruses are mosquito-borne positive-strand RNA viruses belonging to the family of Togaviridae. Chikungunya virus (CHIKV) is the alphavirus causing the largest disease burden worldwide leaving infected people with disabling symptoms that can last for weeks or months and in some rare cases lead to death. The CHIKV, like other alphaviruses, is known to form evaginations at the plasma membrane of infected cells. These structures are called "replication complexes" and are the place where the alphaviral genome gets replicated. The aim of this project is to use cryo-electron tomography to study *in situ* – in human cells - these replication complexes. In order to do so, we culture mammalian cells on electron-microscope grids and transfect these cells with the Chikungunya virus genome in which the structural proteins have been replaced by eGFP. Alternatively, cells are co-transfected with an RNA coding for the structural proteins. By using this approach, we are able to recover viral replicon particles that carry the Chikungunya virus genome without the structural proteins. These viral replicon particles are then used to infect mammalian cells. Infected or transfected cells cultured on grids are then plunge-frozen in order to vitrify them. We are then using cryo-electron tomography and will use subtomogram averaging to get a sub-nanometer structure of these replication complexes and get a better understanding of their organization.

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THE STRUCTURE OF THE MITOCHONDRIAL RIBOSOME FROM *T.THERMOPHILA*

The mitochondrial ribosome has its shares its ancestry with that of the bacterial ribosome but has evolved an overall distinct structure and disparate protein composition. In order to investigate the scope of ribosomal evolution we have previously determined the structure of the mitochondrial ribosome from the ciliate protozoan *Tetrahymena thermophila* at a 3.7 Å overall resolution. The structure revealed a highly divergent ribosome which defied convention with a small subunit with greater mass than the large subunit and harboring at least 26 previously uncharacterized ribosomal proteins. Among the identified ribosomal proteins are enzymes involved in amino acid synthesis, fatty acid catabolism, iron sulfur cluster biogenesis as well as components of the bacterial co-translational protein insertion machinery. In contrast to the overall divergent protein exterior the RNA core of the *T.thermophila* mitochondrial ribosome shows great structural conservation with its bacterial ancestor. The combination of structural conservation and divergence challenges previous observations and hints at the evolutionary path of the mitochondrial ribosome.

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ATOMIC MODEL OF HUMAN MITORIBOSOME

Human mitoribosomes carry out the synthesis of proteins that form part of the oxidative phosphorylation pathway. They vary from their cytosolic counterparts not only in protein and RNA content, but also have specific modifications that are important for stability and correct assembly. We determined the structure of the human mitoribosome at 2.6 Å resolution using single-particle cryo-EM, which allowed visualization of RNA modifications, including base and sugar methylations and pseudouridylations. We confirmed the presence of the previously identified mt rRNA modifications in 12S rRNA of the small subunit (mt-SSU), 16S rRNA of the large subunit (mt-LSU), and mt-tRNA^{Val}. Additional densities might correspond to unreported sites of mt rRNA modifications. Furthermore, from the density map we identified several previously unreported ligands, including nucleotides, polyamines and 2Fe-2S clusters. Three 2Fe-2S clusters observed, were shared between mS25-bS16m, bS6m-bS18m, uL10m-bs18a. Finally, the improved resolution revealed that mS29 at the subunit interface binds ATP rather than GDP as previously suggested, and a new GDP-binding site is identified at a distinct location. Taken together our work represents the most comprehensive description of the structure of human mitoribosome.

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DISCOVERY OF NOVEL UNIVERSAL TRANSLATION FACTOR BY CRYO-EM

Translation regulation is essential for all organisms to respond to the environmental changes, however no universal protein factor has been reported up to date. We purified translating ribosomes from *Plasmodium falciparum* and used extensive focused 3D classification to separate an unknown density complexed with tRNA at the elongation factor binding site. The quality of the data allowed identification of a new factor from the density, namely YchF. This turns out to be a GTPase family member that is universally conserved in all three domains of life, including plastids. The tRNA interactions are base specific only to the CCA end, indicating no specificity to a certain tRNA. Unlike the elongation complex with eEF1a-tRNA, in our structure tRNA is deacylated and has no codon-anticodon interaction, despite presence of mRNA. To confirm the structural data, we performed in vitro translation experiments, which showed that YchF responds to decreased aminoacylation and slows down translation by competing with the other elongation factors on the ribosome. Taken together with the structural data, it suggests that YchF is a putative regulator that involved in avoiding extreme responses, such as inhibition of translation initiation and transcription regulation triggered by deacylated tRNA. In summary, we report a novel universal translation factor identified by cryo-EM.

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VISUALIZING OF PICK1 COMPLEXES AT THE MEMBRANE INTERFACE BY CRYO-ELECTRON MICROSCOPY

PICK1(Protein interacting with C kinase 1) is a modular scaffolding protein containing a PDZ domain, a BAR domain, and an acidic C-terminal tail (ACT). In humans, PICK1 is expressed in all tissues but mostly abundant in brain where it regulates the synaptic targeting and surface expression of many important neuronal proteins, for example, the GluA2 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR). As AMPA receptor trafficking is crucial to excitatory neurotransmission and consequently the synaptic plasticity during long-term potentiation (LTP) and long-term depression (LTD), PICK1 can mediate this activity in the central nervous by interacting with GluR2 through its PDZ domain, and then change the subcellular localization and regulating the amount of GluR2 at plasma membranes by forming co-clusters structures. disruption of this precise regulations will lead to a range of neurological diseases, such as Alzheimer's, Huntington's, and brain ischemia [1]. At present, more than 40 proteins were identified to be interacted with PICK1.

Numerous studies show that PICK1 interacts with diverse proteins through its PDZ while the BAR domain is mainly responsible for curvature sensing and the ACT tail for the negative regulation of binding. However, the mechanism by which PICK1 regulates protein trafficking and how its governing the assembly of complex by different modular on membrane interface remains unclear [2].

In this study, we focus on the complex formed by PICK1 with its interacting partners. We are also showing complexes of PICK1 with the lipid bilayer by Cryo-electron microscopy. We show that PICK1 can bind to curved membranes of lipid nanotubes as well as able to bend membranes by itself. The goal is the generation of a sample amenable for structural interrogation by Cryo-EM. This will help us to elucidate how PICK1 works at the plasma membrane and how PICK1 regulates recruitment of diverse proteins. The structure will provide an avenue for drug design of diseases that involve PICK1.

References:

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MEMBRANE SCAFFOLDS IN SYNAPSES: TOWARDS NOVEL THERAPY AGAINST NEUROLOGICAL DISORDERS

Information processing in the brain is balanced between synapses involved in excitatory and inhibitory signals. Consequences of alterations in this balance are neurological disorders like epilepsy, Alzheimer's, Parkinson's or autism. Although majority of the neural synapses are excitatory, accurate inhibitory signals are important for various learning and adaptive processes. Addiction to alcohol and narcotics or opioids is associated with inhibitory circuits. Pain management also depends on potential activity of inhibitory synapses.

Glycine receptor is a pentameric ion channel and has at least five different alleles thus opening up an overwhelming number of possibilities for each channel, although, all possible conformations are not observed. The receptor has a complex kinetic organisation which includes multiple ligand binding and desensitization steps along with an ion-conductive step in between. The receptor also displays a complex stoichiometry of various alpha (GLRA1-4) and beta subunit (GLRB). The beta subunit has a gephyrin binding motif and forms a scaffold that retains the receptor in the synapse. Although the precise stoichiometry is unclear, the two main possibilities are 2alpha:3beta or 3alpha:2beta. We aim to characterize the channel further, including its various active conformations and establish its stoichiometry using suitable labels. Since the receptor is modulated by alcohol and its ethanol binding sites are characterised, we aim to study the mechanism of geometrical changes upon ethanol binding and develop compounds that displace ethanol and treat alcoholism. We also aim to conduct experiments in the presence of drugs like ethanol and cannabinols.

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REGULATION OF LOCALIZED PROTEIN SYNTHESIS BY NEURONAL RNA GRANULES

The Lab is oriented toward studying the molecular mechanisms underlying the formation, maintenance and reactivation of neuronal RNA granules. Localized mRNAs together with RNA-binding proteins (RBPs) and ribosomes are found in granules, collectively known as neuronal transport RNA granules. The RNA granules serve as the functional units for the transport, storage and activity-dependent translation of synapse-specific mRNAs during localized translation in axodendrites, which is implicated in experience-induced synaptic plasticity. The translational activity within the RNA granules is suppressed during their transport from soma and storage in axodendrites by specific RBPs. Upon synaptic stimulation, this RBP-mediated repression of the granules is relieved by the dissociation of the RBPs and translation follows of the synapse-specific mRNAs. The loss of the RBP-mediated translational silencing underlies numerous neurodegenerative disorders, such as amyotrophic lateral sclerosis/frontotemporal dementia (ALS), spinal muscular atrophy, Huntington's disease, Fragile X Mental Retardation, and autism spectrum disorder. The main interest of the project is to shed a light on structural aspects of RBPs mediated mRNA inactivation in the neuronal RNA granules by using latest advances in the cryo-electron microscopy (cryo-EM), deep-sequencing or mass-spectrometry.

We are using RNA granules extracted from regions of rodent (Wistar rat) brain (hippocampus, cerebellum, or cerebral cortex) implicated in various aspects of memory formation. The RNA granules look morphologically similar to the morula-shaped neuronal RNA granules reported by previous investigators and consist of tightly packed ribosomes. The RNA granule complexes seem to be sensitive to RNase treatment as they fall-apart into monosomes or polysomes after treatment with RNases. Proteomic or Western blot analysis show presence of many RNA binding proteins (RBP) that have previously shown to be part of RNA granules: CAPRIN-1, FMRP, STAU1-2, UPF1, ELAVL1-4, SERBP1, G3BP1, LRRC59, DHX30, YBX1, PUM2, HNRNPU, HNRNPC etc.

We made attempt to resolve single-molecule cryo-EM structure of ribosomes in RNA granules. The RNA granules purified from rat cerebral cortex were used for cryo-EM. The structure (5.5 angstroms) resembles polysomic ribosomes with tRNAs in hybrid A/P and P/E states, mRNA, and nascent chain in peptide exit tunnel. The structure is similar to polysomic pre-translocation complex Rotated-2 state. The results may indicate that most of the ribosomes in RNA granules are stalled polysomic ribosomes as proposed by Graber and colleagues.
To test this theory and understand the binding networks of RBP in RNA granule, we are using cross-linking coupled mass-spectrometry (CL-MS) to identify protein-protein interactions. Protein-protein interactions are proposed to play a dominant role in RNA granule dynamics. The majority of the identified cross-links are between ribosomal proteins as expected. So far, potential interactions (cross-links) between ribosome (r-proteins) and RBPs that we have been able to identify are with rpL7a – HnrnpC, rpL7a – Ncl, rpL30 – Nop2, rpL31 – Btf3, G3BP2 – S28 et al. Further experiments are underway to increase the cryo-EM resolution of the map and identify interaction partners.

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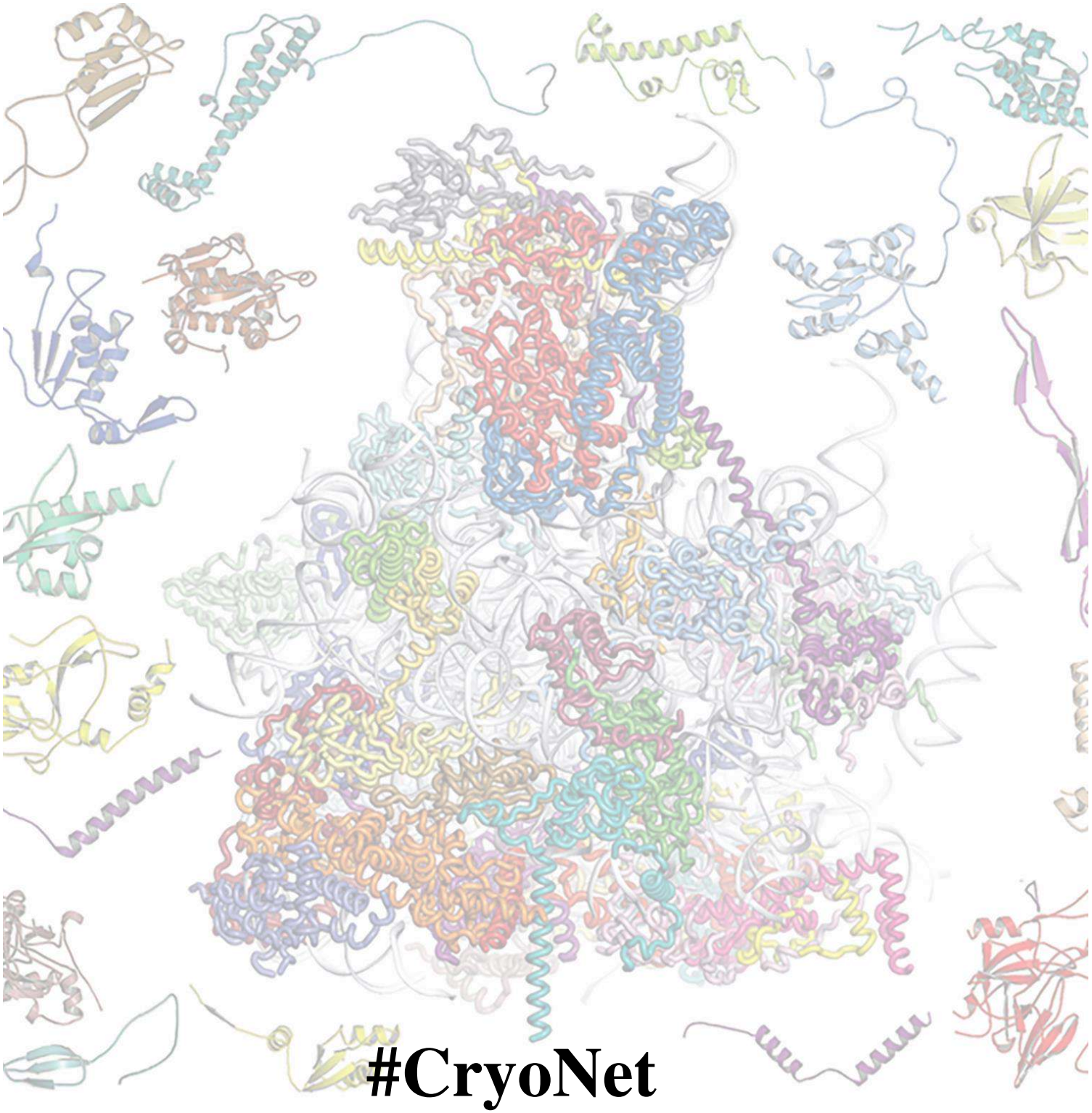
STRUCTURE OF INTACT IMMUNOGLOBULIN E AND THE MECHANISM OF AN ANTI-IGE ANTIBODY REVEALED BY ELECTRON MICROSCOPY

IgE is the central antibody isotype in TH2 biased immunity as well as allergic diseases and autoimmunity. A structural description of the intact antibody is missing but appears as a prerequisite for understanding the contribution of IgE to pathogenesis in allergic diseases. Here we show using electron microscopy, that IgE is a surprisingly rigid molecule that in its major conformation has the Fab arms in fixed positions and limited flexibility in the Fab elbow angles. Furthermore, in this non-symmetric conformation IgE is significantly less bent than predicted from known crystal structures of the Fc fragment alone. Structural comparisons indicate further bending upon association to FcεRI. Binding of the therapeutic antibody ligelizumab to IgE induces an extended unbend conformation with global two-fold symmetry that retains the Fab arms in defined orientations. Based on the deduced epitope and analyses of effector cell activation we show that ligelizumab inhibits IgE binding without displacing receptor bound IgE. Together with the interference of CD23 binding, these data suggest an omalizumab-like function of ligelizumab in agreement with the overlap of their epitopes we observe on IgE. Overall, our data question earlier models of IgE structure-function relationships, and offer an improved structural framework for the treatment of allergic diseases. The observed rigidity of the IgE antibody isotype is unique with major implications for its biology, pathophysiology and therapeutic targeting. These results challenge a paradigm of molecular immunology stating that the flexibility of antibodies is a requirement for their biological function.



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