Tiselius symposium 2025

August 28 2025

Humanities Theatre, Thunbergsvägen 3C, Uppsala

Organizing committee

Erik Marklund

Helena Danielson

Mikael Widersten

Moritz Senger

Sebastian Westenhoff

Ylva Ivarsson

UPPSALA UNIVERSITET

TestaCenter

In collaboration with Cytiva

Invited speakers

Prof. **Lori Passmore**

MRC Laboratory of Molecular Biology, Cambridge, UK – Molecular insights into the mRNA poly(A) tail machinery

Prof. **Markku Varjosalo**

University of Helsinki, Finland – Exploring Protein-Protein Interactions in Disease and Therapy

Prof. **Katja Luck**

Institute of Molecular Biology (IMB), Mainz, Germany – Variant characterization in the intrinsically disordered human proteome

Prof. **Arne Elofsson**

Stockholm University, Sweden – Towards a complete human interactome

Prof. **Richard Neutze**

Gothenburg University, Sweden – Time-resolved serial crystallography of conformational changes in cytochrome c oxidase

Prof. **Katja Petzold**

Karolinska Institutet and Uppsala university, Sweden – Dancing RNA: How RNA structural dynamics is the key to function

Prof. **Philip Ash**

University of Leicester, UK – Electrifying the future of crystallography: combining techniques for mechanistic insight

Dr. **Geoff Holdgate**

ex AstraZeneca, UK – The Value of Traditional Skills: Enzymology & Biophysics in Early Drug Discovery.

Program Tiselius symposium August 28, 2025

Session 1	*Chairs Mikael Widersten & Erik Marklund*
09:00 – 09:05	**Ylva Ivarsson** (Chemistry - BMC, UU, Sweden) Welcome Remarks
09:05 – 09:15	**Helena Danielson** (Chemistry - BMC, UU, Sweden) Tiselius and Biochemistry in Uppsala
09:15-09:20	**Eva Tiselius Bohlin** Memories and reflections as daughter to Arne
09:20 – 09:45	**Lori Passmore** (MRC LMB, Cambridge, UK) Molecular insights into the mRNA poly(A) tail machinery
09:45 – 09:55	**Maria Selmer** (ICM, UU, Sweden) Doublet decoding of tRNASer3 demonstrates plasticity of ribosomal decoding center.
09:55 – 10:20	**Arne Elofsson** (Stockholm University, Sweden) Towards a complete human interactome
10:20 – 10:45	Coffee Break *and poster session (odd numbers)*
Session 2:	*Chair Ylva Ivarsson*
10:45 – 11:10	**Katja Luck** (IMB, Mainz, Germany) Variant characterization in the intrinsically disordered human proteome
11:10 – 11:20	**Laszlo Dobson** (Institute of Molecular Life Sciences, Budapest, Hungary) Insights into pathogen sorting systems with the combination of structural modeling and deep learning
11:20 – 11:30	**Liuqun Zhao** (ICM, UU, Sweden) Δ133p53α and Δ160p53α isoforms of the tumor suppressor protein p53 exert dominant-negative effect primarily by co-aggregation
11:30 – 11:40	**Axel Leppert** (ICM, UU, Sweden) Combining native MS, Mass Photometry, and AI-Based Modeling to capture LLPS-specific interactions.

11:40 – 12:05	**Markku Varjosalo** (University of Helsinki, Finland) Exploring Protein-Protein Interactions in Disease and Therapy
12:05 – 12:10	**Jesper Hedberg** Testa Center/Testa Challenge
12:10 – 13:25	*Lunch Break and poster session (odd and even numbers)*
Session 3:	*Chair Sebastian Westenhoff*
13:25 – 13:50	**Richard Neutze** (University of Gothenburg, Sweden) Time-resolved serial crystallography studies of conformational changes in cytochrome c oxidase
13:50 – 14:00	**Gabriel Ducrocq** (Chemistry – BMC, UU, Sweden) CryoSphere - single particle heterogenous reconstruction from cryoEM
14:00 – 14:10	**Sofia Badolato** (DBB, Stockholm University, Sweden) Gating principles of proton transport in dissected modules of respiratory Complex I
14:10 – 14:20	**Laura Muras** (ICM, UU, Sweden) Deciphering the mechanochemical determinants of nucleosome positioning
14:20 – 14:45	**Katja Petzold** (KI & UU, Sweden) Dancing RNA: How RNA structural dynamics is the key to function
14:45 – 15:15	Coffee Break and poster session (even numbers)
Session 4:	*Chairs Moritz Senger & Helena Danielson*
15:15 – 15:40	**Geoff Holdgate** (ex-AstraZeneca, UK) The Value of Traditional Skills: Enzymology & Biophysics in Early Drug Discovery
15:40 – 15:50	**Guillaume Gaullier** (Chemistry - Ångström, UU, Sweden) Structures of active and inactive carboxysomal carbonic anhydrase
15:50 – 16:00	**Kushal Sengupta** (Max Planck Institute for Chemical Energy Conversion, Mülheim an der Ruhr, Germany)

	Understanding the Reaction Mechanism of Vanadium Nitrogenase: Insights from Spectroscopy and Biochemistry
16:00 – 16:25	**Philip Ash** (University of Leicester, UK) Electrifying the future of crystallography: combining techniques for mechanistic insight
16:25 – 16:30	**Sebastian Westenhoff** (Chemistry – BMC, UU, Sweden) Closing Remarks *End*

Molecular insights into the mRNA poly(A) tail machinery

Lori Passmore

MRC, LMB, Cambridge, UK

Cytoplasmic shortening of mRNA poly(A) tails represses eukaryotic gene expression by inhibiting efficient translation and committing an mRNA to decay. This occurs in gene- and context-dependent manners to allow transcript-specific control of poly(A) tail lengths. Understanding how specific mRNAs are targeted for deadenylation has been a central question in the field of gene expression for many years. Specificity is achieved through RNA adaptors – RNA-binding proteins that recruit the CCR4-NOT deadenylase machinery to certain substrate mRNAs in a regulated manner to target specific transcripts for deadenylation. Examples of RNA adapters are Pumilio/Puf proteins, TTP/tristetraprolin and the microRNA-induced silencing complex (miRISC). On the other hand, the PAN2–PAN3 deadenylation complex is generally thought to act non-discriminately on long poly(A) tails. Still, the molecular mechanisms of specificity remain unclear in many cases. We use structural biology and biochemical reconstitution to approach this problem. We find that RNA adapters are much more widespread than previously thought. Second, we find that RNA adapters interact with CCR4-NOT via multivalent interactions with their intrinsically disordered regions (IDRs). Finally, these interactions can be regulated to mediated tuning of deadenylation rate. Together, this contributes to the intricate regulation of gene expression.

Doublet decoding of tRNASer3 demonstrates plasticity of ribosomal decoding center

Shruthi Krishnaswamy, Shirin Akbar, Daniel Larsson, Yang Chen & Maria Selmer
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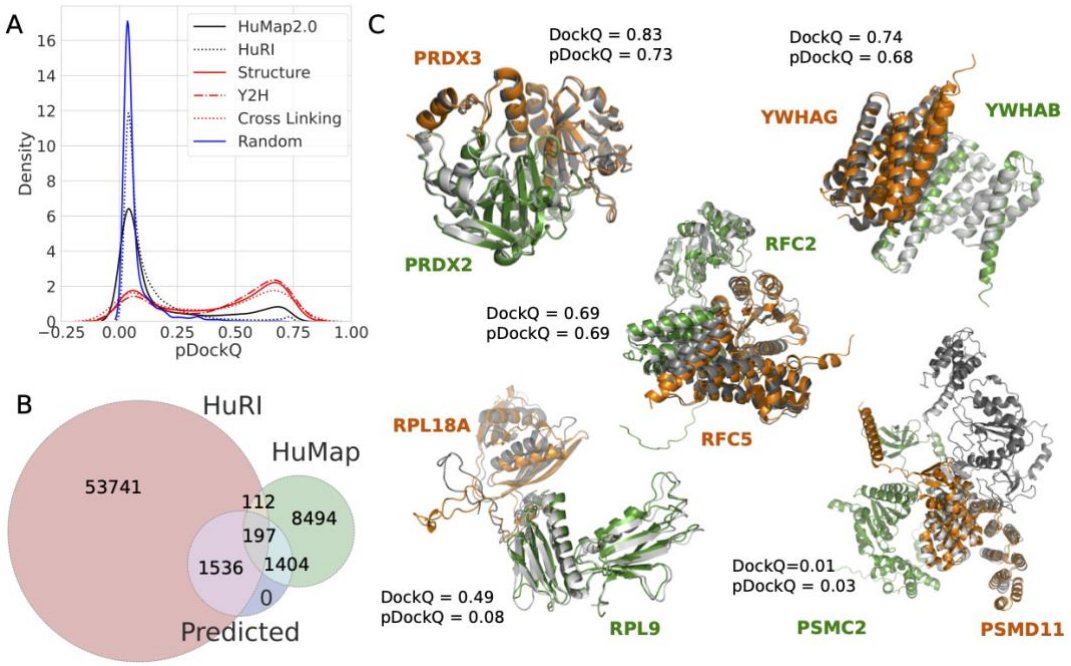
The doublet decoding hypothesis was proposed to explain how two wildtype tRNAs, the AGC-decoding tRNASer3 and the CCA-decoding tRNAThr3 with low frequency could induce -1 frameshifts on non-cognate GCA and GGA codons in E. coli, by forming only two codon-anticodon base pairs (Atkins et al., 1979). It has remained unclear whether this non-standard decoding can occur on the ribosome. To test the doublet-decoding hypothesis, we performed single-particle cryo-EM reconstructions on E. coli 70S ribosomes with the frameshift-inducing tRNASer3 bound to the non-cognate GCA codon or the cognate AGC codon in the ribosomal A site. Our structures confirm that doublet decoding occurs when the GCU anticodon of tRNASer3 forms only two Watson-Crick base pairs between anticodon bases G34 and C35 and the two first bases of the GCA alanine codon. This interaction is made possible by U36 from the anticodon forming a Hoogsteen base pair with monitoring base A1493, pushing the first base of the A-site codon in position for interaction with C35 of the anticodon. Nitrocellulose filter-binding experiments show that the Ser3 anticodon stem-loop (ASL) binds to the A-site GCA codon with eight-fold lower affinity compared to the cognate ASL, a similar difference as between ASLPhe binding to a fully complementary UUC codon or a UUU codon with a third-position wobble base pair. The results demonstrate how doublet-decoding exploits the plasticity of the decoding center.

Towards a complete human interactome

Arne Elofsson

DBB and SciLifeLab, Stockholm University

Cellular functions are governed by molecular machines that assemble through protein-protein interactions. Their atomic details are critical to studying their molecular mechanisms. Today the structure of virtually all individual proteins is available from predictions using AlphaFold. However, these predictions are limited to individual chains and do not include interactions. In this talk I will describe our attempts to increase the structural coverage of protein-protein interactions. Today fewer than 5% of hundreds of thousands of human protein interactions have been structurally characterised. We show that combining predictions and experiments can orthogonally confirm higher-confidence models, and using AlphaFold2, we have built 3,137 high-confidence models, of which 1,371 have no homology to a known structure. We are exploring rapid methods to identify protein interaction networks. Finally, we show how the predicted binary complexes can be used to build very larger assemblies using a Monte Carlo Tree search method.



Variant characterization in the intrinsically disordered human proteome

Katja Luck

IMB, Mainz, Germany

Protein-protein interactions (PPIs) are being mapped experimentally at increasing throughput. However, HTP PPI assays can only tell us which proteins interact with each other but not how. This lack in structural information massively hinders inference of physiological and pathological molecular mechanisms. The structural diversity by which proteins can interact with each other is immense and largely driven by intrinsically disordered regions (IDRs) in proteins that mediate transient signaling and regulatory PPIs. Many identified disease-associated human variants fall in IDRs and remain uncharacterized because neither positional sequence conservation nor stable protein structure can be used to predict their effects on protein function. Structures of PPI interfaces involving IDRs would advance variant effect prediction, however, we find that computational tools such as AlphaFold struggle to accurately predict these modes of protein binding. Using in-house developed pipelines we generate high confident structural models of 3500 disorder-containing PPI interfaces and identify more than 1000 uncharacterized variants in disordered interfaces that are likely disruptive to protein binding. Our predictions are supported by experimental validations of selected predicted interfaces and variant effects providing a clear avenue towards system-wide incorporation of structural information for variant effect prediction in IDRs.

Insights into pathogen sorting systems with the combination of structural modeling and deep learning

Andras Zeke[1], Zsofia E. Kalman[2], Rita Pancsa[3], Gabor Tusnady[3,4], Toby Gibson[5] & Laszlo Dobson[3,4]

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The pathogenic, tropical Leishmania flagellates belong to an early-branching eukaryotic lineage (Kinetoplastida) with several unique features. Unfortunately, they are poorly understood from a molecular biology perspective, making development of mechanistically novel and selective drugs difficult. We show critical targeting short linear motif systems as well as their receptors in depth, using a combination of structural modeling, evolutionary sequence divergence and deep learning. Secretory signal peptides, endoplasmic reticulum (ER) retention motifs (KDEL motifs) are ancient and essential components of cellular life. Although expected to be conserved amongst the kinetoplastids, we observe that all these systems show a varying degree of divergence from their better studied equivalents in animals, plants, or fungi. We not only describe their behaviour, but also build models that allow the prediction of localization and potential functions for several uncharacterized Leishmania proteins. Furthermore, we demonstrate how an entirely different pathogen sorting system in Plasmodium falciparum can serve as a guide to identify secreted Leishmania proteins with the potential ability to enter the host cytoplasm and tamper with critical regulatory and innate immunity mechanisms.

Δ133p53α and Δ160p53α isoforms of the tumor suppressor protein p53 exert dominant-negative effect primarily by co-aggregation

Liuqun Zhao[1], Tanel Punga[2], Suparna Sanyal[1]
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Background: p53 is a tumor suppressor protein with multiple isoforms with shared or specific functions. However, two of its isoforms, Δ133p53α and Δ160p53α, with large N-terminal deletions, can cause cancer. These isoforms exert a dominant-negative effect on full-length p53 (FLp53), although the precise molecular mechanisms are unknown.
Aim: Investigate molecular mechanisms underlying dominant-negative effect of FLp53 by Δ133p53α and Δ160p53α Isoforms.
Methods: The research utilized a combination of experimental techniques, including chromatin immunoprecipitation, luciferase expression, subcellular fractionation, immunofluorescence assays, and apoptotic caspase activity assay, to explore the behavior of the p53 isoforms.
Results: The findings elucidate that these DNA-binding deficient p53 isoforms form hetero-tetrameric complexes with FLp53 and disrupt FLp53's DNA binding and transcriptional activities when present in a higher proportion than FLp53 in the tetramer. However, these structurally unstable isoforms promote vigorous protein aggregation involving FLp53, disrupting its structure and sequestering it in the cytoplasmic and nuclear aggregates, thereby limiting its availability to function as a transcription activator protein.
Conclusions: Co-aggregation of Δ133p53α and Δ160p53α with FLp53, rather than hetero-tetramerization, is likely the primary factor contributing to their dominant-negative effect. Modulating the stability and aggregation of p53 isoforms could be a novel strategy for cancer therapy.

Combining native MS, Mass Photometry, and AI-Based Modeling to capture LLPS-specific interactions.

Hannah Osterholz[1], Gefei Chen[1,2], Michael Landreh[1,2] & Axel Leppert[1]
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Tau is an intrinsically disordered microtubule-associated protein that undergoes liquid–liquid phase separation (LLPS), a process implicated in both normal cellular function and neurodegenerative disease. Mechanistic understanding of tau LLPS and its regulation remains limited, partly due to the dynamic and heterogeneous nature of such condensates and the difficulty of resolving interactions with binding partners under these conditions. Here, we present an integrative approach combining native mass spectrometry (MS), mass photometry (MP), and AI-based structural modeling to dissect tau conformations and binding of LLPS-specific modulators under phase-separating conditions.

We developed a native MS-compatible assay to study tau LLPS, revealing that tau undergoes conformational compaction under phase-separating conditions, enableing selective binding of small molecules. This suggests that tau adopts specific conformations or transient interfaces under LLPS conditions that are competent for interaction. To probe how protein binding partners influence the tau interaction landscape during LLPS, we used tubulin as a model substrate known to assemble within tau condensates. Mass photometry confirmed complex formation between tau and tubulin under LLPS conditions. Next, we introduced the molecular chaperone domain BRICHOS, derived from the extracellular protein Bri2, to test whether this assembly can be modulated. BRICHOS also partitions into tau condensates and binds to tau, reducing tau–tubulin complex formation. Mass spectrometry and AlphaFold modeling indicates that BRICHOS and tubulin share an overlapping binding site on tau, suggesting a competitive mechanism of interaction.

Together, our results demonstrate that combining native MS, MP and an AI-based modeling strategy enables characterization of the dynamic behavior of intrinsically disordered proteins and offers a powerful framework for investigating biomolecular condensates.

Exploring Protein-Protein Interactions in Disease and Therapy"

Markku Varjosalo
University of Helsinki, Finland

Protein-protein interactions (PPIs) are central to cellular signaling networks and their disruption underlies numerous human diseases. Systematic identification and characterization of these interactions can reveal critical insights into disease mechanisms. Our research employs quantitative mass spectrometry-based proteomics combined with computational biology to map PPIs involving essential signaling proteins, including protein kinases, phosphatases, and transcription factors. We focus particularly on understanding how these interactions drive disease progression in immunological disorders and cancer, identifying novel therapeutic targets. Additionally, our structural proteomics approaches have enabled detailed characterization of disease-relevant protein complexes, exemplified by recent insights into the architecture and function of the Commander complex.

Time-resolved serial crystallography studies of conformational changes in cytochrome *c* oxidase

Richard Neutze

Other contributing authors: Doris Zorić, Jonatan Johannesson, Emil Sandelin, Adams Vallejos, Arpitha Kabbinale, Swagatha Ghosh, Gisela Brändén

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Time-resolved X-ray crystallography allows time-dependent structural changes to be visualized as they evolve within protein crystals and can yield unique structural insights into the course of a biochemical reaction (*1*). Serial crystallography (*2*) is now routinely used for time-resolved X-ray diffraction studies of macromolecules at X-ray free electron laser and synchrotron radiation facilities. As the field grows, biological reactions that are not naturally light sensitive will be increasingly studied using time-resolved serial crystallography (*1*). In this work, a laser-flash was used to release photocaged oxygen and thereby initiate the reduction of oxygen to water in microcrystals of cytochrome *c* oxidase (*3*). Our structural results reveal how this reaction may be coupled to gating the uptake of protons from the cytoplasm and to the release of protons to the periplasm.

References:

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3. R. Andersson *et al.*, Serial femtosecond crystallography structure of cytochrome c oxidase at room temperature. *Sci Rep* **7**, 4518 (2017).

CryoSphere - single particle heterogenous reconstruction from cryoEM

Gabriel Ducrocq*, Lukas Grunewald*, Sebastian Westenhoff*, Fredrik Lindsten**
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***Division of Statistics and Machine Learning, Linkoping University, Linköping, Sweden*

The three-dimensional structure of proteins plays a crucial role in determining their function. Protein structure prediction methods, like AlphaFold, offer rapid access to a protein structure. However, large protein complexes cannot be reliably predicted, and proteins are dynamic, making it important to resolve their full conformational distribution. Single-particle cryo-electron microscopy (cryo-EM) is a powerful tool for determining the structures of large protein complexes. Importantly, the numerous images of a given protein contain underutilized information about conformational heterogeneity. These images are very noisy projections of the protein, and traditional methods for cryo-EM reconstruction are limited to recovering only one or a few consensus conformations. In this paper, we introduce cryoSPHERE, which is a deep learning method that uses a nominal protein structure (e.g., from AlphaFold) as input, learns how to divide it into segments, and moves these segments as approximately rigid bodies to fit the different conformations present in the cryo-EM dataset. This approach provides enough constraints to enable meaningful reconstructions of single protein structural ensembles. We demonstrate this with two synthetic datasets featuring varying levels of noise, as well as two real dataset. We show that cryoSPHERE is very resilient to the high levels of noise typically encountered in experiments, where we see consistent improvements over the current state-of-the-art for heterogeneous reconstruction.

Gating principles of proton transport in dissected modules of respiratory Complex I

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The respiratory Complex I is the first enzyme of respiratory chains that powers energy transduction in the cell. Complex I transfers electrons from NADH to a quinone molecule and couples these reactions to the pumping of four protons across the inner mitochondrial membrane, and to power the synthesis of ATP. Yet, despite its fundamental role, and its association to several mitochondrial diseases, the molecular mechanism of Complex I still remains highly debated. To study the key principles of the long-range proton pumping, we dissect here the antiporter-like subunit of the membrane domain of Complex I to study their proton conduction by combing time-resolved spectroscopy, site-directed mutagenesis, and multiscale molecular simulations. We identify key gating residues that modulate the proton conduction in proteoliposomes. Our combined findings provide new insights into the elusive long-range energy transduction mechanism of Complex I and to molecular principles underlying ion transport across biological membranes.

Deciphering the mechanochemical determinants of nucleosome positioning

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Life is defined by dynamic self-organization across molecular to cellular scales. Nowhere is this more evident than in the organization of genetic material, where the functional state and spatial arrangement of DNA are interdependent through chromatin, a condensed complex of DNA and associated proteins. At its core, chromatin structure is shaped by ATP-dependent remodeling enzymes that reposition nucleosomes - the basic units of chromatin - to regulate DNA accessibility for gene expression and genome stability. Yet the dynamic mechanisms and reaction pathways by which these molecular machines interpret DNA sequence-dependent cues and extranucleosomal factors in the crowded chromatin environment remain largely unresolved. While ensemble and endpoint assays reveal static nucleosome distributions, they fail to capture the dynamic, force-sensitive behaviors critical for remodeler function in vivo. Current high-resolution methods also often lack the range needed to fully resolve nucleosome positioning mechanisms. To address these limitations, I present a novel single-beam optical tweezers assay that enables real-time tracking of nucleosome sliding under single-molecule conditions, with near-base-pair spatial resolution across extended DNA distances. This approach directly captures key parameters underlying nucleosome patterning - including sliding rates, processivity, direction switching, and force sensitivity of remodeling enzymes. I will discuss our initial experiments with the yeast remodeler Chd1, through which I systematically dissect how DNA sequence and the presence of roadblocks influence the magnitude and directionality of nucleosome sliding. This method enables comprehensive studies of the dynamic features and mechanochemical determinants of chromatin remodeling, offering mechanistic insight into chromatin organization, gene regulation, and diseases linked to aberrant remodeling.

Dancing RNA: How RNA structural dynamics is the key to function

Katja Petzold & members of the Petzold Lab[1] & A. Chen Lab[2]

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Many functions of RNA depend on rearrangements in secondary structure that are triggered by external factors, such as protein or small molecule binding. These transitions can feature on one hand localized structural changes in base pairs1 or can be presented by a change in the chemical identity of e.g. a nucleo-base tautomer2. We use and develop R1ρ-relaxation-dispersion NMR methods3,4 for characterizing transient structures of RNA that exist in low abundance (populations <10%) and that are sampled on timescales spanning three orders of magnitude. We further analyse RNA complexes using RNA structural probing5, Cryo-EM and functional assays of the trapped states.

Two different systems are going to be discussed: 1) A microRNA – mRNA complex changes conformation to activate the RISC complex6 and how microRNAs in general select their targets7. 2) Flipping the 3D stabilizer A-minor motifs to regulate ribosome dynamics. Furthermore an outlook on recent efforts to measure in-cell NMR of nucleic acids in functional complexes8will be given.

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3) Steiner, E., Schlagnitweit, J., Lundström, P. & Petzold, K. Capturing Excited States in the Fast-Intermediate Exchange Limit in Biological Systems Using 1H NMR Spectroscopy. Angewandte, (2016)

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The Value of Traditional Skills: Enzymology & Biophysics in Early Drug Discovery

Geoff Holdgate
ex-AstraZeneca, UK

In the continually evolving landscape of drug discovery, traditional skills such as enzymology and biophysics remain pivotal in target understanding, hit identification and the hit-to lead process. This talk highlights the critical role these disciplines play in identifying, validating, and optimizing drug candidates. An overview of the early drug discovery process highlights their contribution in the pre-clinical phases.

The fundamental importance of enzymology, the study of enzymes as biological catalysts, in understanding drug interactions at a molecular level, and the contribution of biophysics, which explores the physical principles underlying biological systems, providing essential insights into the dynamic behavior of drug targets, are discussed and their impact highlighted.

The presentation includes five recent, brief exemplar case studies that underscore the importance of these traditional skills in designing and understanding assays and compound behaviour, as well as highlighting the importance of data analysis, interpretation, and exploitation. Attendees will gain an appreciation of how these tried-and-tested approaches continue to drive innovation in developing safe and effective medicines.

Structures of active and inactive carboxysomal carbonic anhydrase

Guillaume Gaullier, Nikoleta Vogiatzi, Cecilia Blikstad
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Cyanobacteria contribute significantly to primary production, achieving high CO2 fixation rates via their carbon concentration mechanism (CCM). The carbonic anhydrase (CA) is a critical part of the CCM and must be tightly regulated. Cytosolic CA activity would "short-circuit" the CCM, as CO2 produced in the cytosol would diffuse out of the cell. How the CA is encapsulated into carboxysomes via interaction with rubisco is now well understood (Blikstad et al, 2023). How it is kept inactive in the cytosol and activated inside carboxysomes is still not known.
We determined cryoEM structures of active and inactive CA from the same protein preparation used in activity measurements. CA is a trimer of dimers regardless of activity, indicating oligomerization likely plays no role in regulation. 3D classification of the dimers found two distinct global conformations in both the active and inactive conditions, and local conformational differences close to the active site, revealing hallmarks of the active state. A structure of a mutant CA devoid of activity in the condition permissive for the wild-type enzyme's activity further shows that some structural determinants of activity are independent of each other, and that all are required for activity. Our findings show that CA activity is regulated by its chemical environment through global and local conformational changes, offering insight for engineering a functional cyanobacterial CCM in C3 chloroplasts.
Through collaborations, we also recently determined cryoEM structures of other photosynthetic and CCM complexes from cyanobacteria and plant.

Understanding the Reaction Mechanism of Vanadium Nitrogenase: Insights from Spectroscopy and Biochemistry

Isis M Wahl, Nina Breuer, Kushal Sengupta* and Serena DeBeer*
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Vanadium nitrogenase (V-N2ase) is a key member of the nitrogenase family of enzymes, catalyzing the ATP-dependent reduction of dinitrogen to ammonia. While it shares a common two-component (catalytic MFe and reductase protein) architecture with its molybdenum-dependent counterpart (Mo-N2ase), significant differences in cofactor/protein structure, electronic properties, and reactivity set it apart. V-N2ase not only displays broader substrate scope, including CO, but also features distinct catalytic rates and redox behavior, suggesting a mechanistic divergence from the well-studied Mo-N2ase. At the molecular level, the VFe protein hosts the FeV cofactor (FeVco) and a P-cluster (Fe_8S_7) responsible for electron transfer. Unlike MoFe which has a well-characterized S = 3/2 signal from FeMoco, VFe exhibits a more complex EPR signature. Notably, both holo- and apo-VFe samples show persistent S = 1/2 signals, attributed to $[Fe_4S_4]$-like fragments of the P-cluster, alongside S = 3/2 and S = 5/2 signals believed to arise from FeVco. Interestingly, the relative population of these spin systems varies with the time point at which cell cultures are harvested—highlighting the sensitivity of the protein's electronic structure to biochemical preparation and expression conditions. These features suggest structural and electronic heterogeneity, possibly reflecting distinct cofactor maturation pathways and redox flexibility.To better understand the mechanism of VFe, our group has combined varied biochemical sample preparation with advanced spectroscopic characterization. Alongside understanding the resting state, by modulating electron flux during turnover conditions, we have been studying early catalytic intermediates—most notably the E_1 state—and monitored their formation using both Fe and V K-edge X-ray absorption spectroscopy (XAS) and EPR. Our XANES data reveal Fe-centered redox changes with no evidence for a redox event at vanadium and only subtle changes observed in the V K-pre-edge region, consistent with minor modulations in the local coordination environment. In parallel, EXAFS data demonstrate structural rearrangements in both Fe and V shells, supporting changes in cofactor geometry during catalysis.

Electrifying the future of crystallography: combining techniques for mechanistic insight

Philip Ash (University of Leicester, UK)

POSTER ABSTRACTS

1. A Biosensor Toolbox for Monitoring Recombinant Protein Production in Escherichia coli

Maurice Mürköster[1], Mateusz Balka[1], Christina J. Kamp[1], Erik Tunäng[1], Hanna-Louise Ögren[1], Silvia Theopold[1], Alister J. Cumming[1], Alex Toftgaard Nielsen[2] and Daniel O. Daley[1]
[1] Department of Biochemistry and Biophysics, Stockholm University, SE-19468, Sweden.
[2] The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, 2800 Kongens Lyngby, Denmark.

Herein we describe a collection of biosensor plasmids (a toolbox) for monitoring recombinant protein production in Escherichia coli. The plasmids are activated by OFF-pathway events, such as (1) protein aggregation in the cytoplasm, (2) bottlenecks at the Sec translocon, and (3) protein aggregation in the periplasm. Each plasmid was constructed using a fast-folding and fast-degrading version of the Green Fluorescent Protein so that OFF-pathway events can be detected in vivo and in real-time, by simply monitoring whole cell fluorescence. The toolbox will be a valuable resource for diagnosing protein production problems in E. coli, evaluating solutions that mitigate these problems, and building self-regulating genetic circuits that avoid production problems. The toolbox is available through Addgene.

2. Turning green when things go wrong: A biosensor toolbox for monitoring recombinant protein production in E. coli

Maurice Mürköster[1], Mateusz Balka[1], Christina J. Kamp[1], Erik Tunäng[1], Hanna-Louise Ögren[1], Silvia Theopold[1], Alister J. Cumming[1], Alex Toftgaard Nielsen[2] and Daniel O. Daley[1]
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Cellular stress is caused by OFF-pathway events and a key factor influencing the yield and quality of recombinant proteins. Too much stress can lead to reduced growth, protein degradation or even cell death, making it imperative to reduce or avoid stress during recombinant protein production. To address this, we developed a biosensor toolbox designed to detect OFF-pathway events during recombinant protein production in Escherichia coli. This toolbox includes biosensors that monitor protein aggregation in the cytoplasm and periplasm, as well as bottlenecks in the Sec translocon. Each biosensor expresses a fast-folding and fast-degrading variant of green fluorescent protein (GFP), allowing for a simple real-time detection by measuring whole-cell fluorescence. We validated the biosensors in this toolbox systematically using model proteins known to either aggregate or fold properly, with additional testing of Sec and periplasmic sensors using proteins with or without Sec-dependent targeting sequences. All biosensors showed specific activation in response to their intended stress condition and remained inactive otherwise which was also confirmed by SDS-PAGE and Western blot analysis. Our toolbox offers a simple and user-friendly method for identifying cellular stress during recombinant protein production. The insights this toolbox provides can be used during process optimization and research into protein folding, cellular stress and quality control, making it a valuable tool for both industrial applications and basic research.

3. Structural mechanism of FusB-mediated rescue from fusidic acid inhibition of protein synthesis

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The antibiotic resistance protein FusB rescues protein synthesis from inhibition by the antibiotic fusidic acid (FA), which locks elongation factor G (EF-G) to the ribosome after GTP hydrolysis. Here, we present time-resolved single-particle cryo-EM structures explaining the mechanism of FusB-mediated rescue. FusB binds to the FA-trapped EF-G on the ribosome, causing large-scale conformational changes of EF-G that break interactions with the ribosome, tRNA, and mRNA. This leads to dissociation of EF-G from the ribosome, followed by FA release. We also observe two independent binding sites of FusB on the classical-state ribosome, overlapping with the binding site of EF-G to each of the ribosomal subunits, yet not inhibiting tRNA delivery. The direct interaction between FusB and the S. aureus ribosome was characterized with fluorescence polarization, showing a KD of 320 □ 31 nM. Relative quantification of FusB and EF-G in a clinical S. aureus isolate with mass spectrometry resulted in a FusB:EF-G ratio of 1:2.3□0.3. These data together support that direct binding of FusB to ribosomes could occur in the cell, but its function remains unclear. Our results reveal an intricate resistance mechanism involving specific interactions of FusB with both EF-G and the ribosome, and a non-canonical release pathway of EF-G.

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4. Ribosomal RNA modifications around the peptidyl transfer center stimulate catalytic activity and prevent formation of alternative structures

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The catalytic site of the ribosome, the peptidyl transferase center (PTC), is entirely composed of rRNA and is decorated by modified nucleotides. The functional and structural importance of these modifications has remained obscure. Here, we demonstrate that E. coli ribosomes lacking 12 or 13 modifications around the PTC can catalyze peptide bond synthesis, but at a slower rate than WT ribosomes. In vitro peptide bond synthesis is characterized by increased entropic penalty and a reduction in the enthalpy, which can be explained by weaker substrate binding. In addition, hypo-modified ribosomes are also prone to inactivation at elevated temperatures. To understand the structural importance of these post-transcriptional modifications, we solved high-resolution cryo-EM structures of hypo-modified ribosomes in complexes with mRNA, A-site, and P-site tRNA. We found numerous alternative structures of the rRNA in the PTC region and of r-protein uL4. Many residues essential for substrate binding and catalysis are displaced and the geometry of the nascent chain exit tunnel is affected. We observe a strong inherent propensity of hypomodified rRNA to form alternatively stacked structures. The redundant nature of rRNA modifications can be explained by multiple post-transcriptional modifications working in unison to stabilize the WT conformation and prevent non-native contacts. Misfolded PTC-structures explain the

weaker substrate affinity, reflected in reduced tRNA occupancy and misfolding of the CCA-end of A-site tRNA. The modifications are thus important to stabilize the active native fold of the PTC and to prevent misfolded conformations.

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5. AphaFold-Based Prediction of Viral SLiM-Mediated Hijacking of Innate Immune Signaling

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Despite major advances in mapping the human interactome with techniques such as mass spectrometry and yeast two-hybrid screening, many transient and low-affinity interactions remain elusive. These often involve short linear motifs (SLiMs) located within intrinsically disordered regions, whose weak binding makes them difficult to detect experimentally. Recent work has shown that AlphaFold can capture SLiM-mediated interactions with notable accuracy. Building on this, we systematically compare different AlphaFold scoring functions and establish a screening strategy designed to overcome its limitations, enabling robust identification of novel SLiMs. We apply this workflow to the JAK–STAT pathway in both human and viral systems, uncovering multiple SLiM interactions, including a previously uncharacterized STAT2–STAT5 binding motif. Our approach highlights key interaction sites and novel binding partners, offering new insights into cellular regulation and potential therapeutic targets.

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6. Exploring the effect of alternative splicing on motif-based protein–protein interactions

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Short linear motifs (SLiMs) mediate many of the key protein–protein interactions (PPIs) within the cell. The SLiM-based interactome is predicted to differ substantially between isoforms of the same gene, with each isoform pair sharing only a fraction of their interaction partners. However, experimental evidence on the extent to which alternative splicing perturbs PPI networks is limited. Here, we systematically investigate how alternative splicing rewires motif-based PPIs by generating an M13 bacteriophage library tiling the intrinsically disordered regions (IDRs) of 97,000 alternative isoforms from 11,401 human genes. Using Proteomic Peptide Phage Display (ProP-PD), we screened the phage library against 192 bait domains from ~40 domains, yielding the first experimentally derived SLiM-based interactome for alternative isoforms. Ongoing analyses will quantify the frequency of motif-breaking and motif-making splicing events, evaluate changes in motif flanking regions and their effects on interaction affinities, as well as provide insight into the functional impact of isoform switching.

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7. Large-scale phage-based profiling of E3-ligase reveals binding motifs, interactome and potential novel degrons

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Ubiquitination is a post-translational signaling event that coordinates not only protein homeostasis but also many different cellular responses such as DNA damage control or the cell cycle. An estimated 600 to 1,000 different E3-ligases orchestrate these signals by identifying and ubiquitinating the correct substrates. Many E3-ligases are targeted to their substrates by binding short linear motifs (degradation motifs, degrons), well known examples include MDM2 binding to a FxxxWxxL motif in p53, KEAP1 binding to a TGE motif in NFE2L1 and WW domains of HECT type E3 ligases binding to PPxY motifs. However, the specificity determinants of the binding motifs remain unknown for many E3-ligases. In this study we investigate the SLiM-based interactions of over 100 domains E3-ligases using proteomic-peptide-phage display (ProP-PD), which enables the screening of peptide ligands among one million peptides from the human proteome. The phage-based screening uncovered over thousand novel E3 ligase-peptide interactions. Based on the results, known and novel binding motifs were established. For example, novel ligands of the PUB domain of RNF31 were identified. Interactions are being validated using a panel of computational, biophysical and cell biological approaches. Finally, cell sorting experiments were attempted evaluate the degron-capacity of the discovered peptide binders. The results will shed novel light on the motif-based interactions that target E3 ligases to complexes.

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8. Cooperation of SH3 domains in ligand binding and autoinhibition

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SH3 domains are among the most widespread domain families in the human proteome and exhibit high versatility in binding proline-rich regions of proteins. SH3 domains can not only function as single binding-modules, but can also cooperate to form a binding cleft. The most well-characterized example is the p47phox protein, in which tandem SH3 domains bind an internal "APPRR" motif (autoinhibition) and also interact with a p22phox-derived peptide. In addition to p47phox, members of the related superfamily—such as TKS4—have been hypothesized to form similar autoinhibitory complexes.

To our knowledge, cooperativeness of tandem SH3 domains binding outside this protein family has not yet been systematically investigated. This may be due in part to the experimental challenges posed by such systems. Using bioinformatic tools, we have identified additional candidate proteins in which SH3 domains may function cooperatively, forming a binding cleft for partner binding and contributing to autoinhibition. Beside identifying new cases, we also characterize the molecular features of the tandem SH3 domains, and also describe candidate motifs recognized by them.

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9. Evolution of structure and binding motifs in naturally-evolved human *de novo* proteins

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In recent years, it has become apparent that emergence of de novo genes and their protein products is common across all cellular life. The advent of ribosome profiling has revealed that previously non-coding regions can gain short open reading frames (sORFs) which may encode putative novel proteins. This poses the question of how these novel proteins, hitherto unseen to evolution, may become integrated into cellular networks and possibly acquire novel functions. To answer this question, we selected 30 putative human sORF proteins from the sORF database and studied their structure and potential for interacting with cellular proteins. We found that sORFs are primarily disordered, but have varying degrees of helical propensity, suggesting some may fold upon binding to a partner in the cell. We then searched the sORF sequences for short linear motifs recognized by cellular proteins and identified several candidate interaction partners, which were validated with fluorescence polarization affinity measurements. Our data show that sORFs can evolve motifs recognized by various cellular proteins. Herein, we present one of the few experimental characterizations of the structure and binding potential of human sORFs.

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10. Native Mass Spectrometry Captures the Conformational Plasticity of Proteins with Low-Complexity Domains

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Membraneless organelles have a wide range of functions and typically form through liquid–liquid phase separation (LLPS) of proteins with intrinsically disordered low-complexity (LC) domains. However, the combination of structured and disordered regions makes studying these proteins extremely challenging. Native mass spectrometry (nMS) with nano-electrospray ionization (nESI) is a powerful method for analyzing the conformations of folded, disordered, and partially disordered proteins by transferring them from solution into the gas phase. The number of charges that folded proteins acquire during nESI scales with their surface area, while in disordered proteins, the number of charges is related to the number of exposed basic residues that become protonated. LC domains are disordered but lack ionizable residues, raising the

question of whether their charging pattern reflects the protein's folded state. To address this question, we analyzed the charge state distribution of designed proteins with a structured N-terminal domain connected to different LC domains. Interestingly, the proteins ionize in distinct charge state distributions that correlate with their degree of disorder in solution. The correlation holds true also for variants with no ionizable residues in the LC domain, allowing us to predict the disorder content regardless of their sequence. Applying this method to naturally occurring protein complexes, we can use charge state signatures to assign different conformational states to their intrinsically disordered regions.

In summary, we demonstrate that native MS can be used to obtain structural insights into proteins containing both ordered and disordered regions, even in the absence of ionizable residues. Our study highlights the potential of nMS in exploring the structural dynamics of phase-separating proteins and their role in LLPS function.

List of participants

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11. Thermal activation of protein complexes in the gas phase: Insights from molecular dynamics simulations

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Native mass spectrometry and ion mobility are powerful techniques for probing the structures and interactions of proteins. These techniques rely on collisions with inert gas molecules, which can be tuned to induce either gentle thermal activation through low-energy collisions or more substantial structural changes with high-energy collisions. While the effects of high-energy activation are relatively well characterized, the mechanisms driving structural changes at low-to-moderate activation energies remain poorly understood.

In this study, we molecular dynamics simulations and complementary experiments to investigate protein structural dynamics across a range of collisional activation conditions. Our results uncover how collisional energy modulates the delicate balance between compactive and expansive forces within protein ions, leading to distinct conformational transitions. These insights not only deepen our understanding of gas-phase protein behavior but also enhance the interpretive power of native mass spectrometry and ion mobility measurements in structural biology.

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12. Insights into phytochrome dark reversion mechanism from cryo-EM and computation

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Light-driven processes are widely recognized as the foundation to signaling cascades essential for sustaining life. Light-transducing proteins like phytochromes are the starting point of this mechanism and thus bare a highly complex machine propagating small conformational changes via the whole structure activating the following reaction interface. Phytochromes function as photoswitches reacting to different wavelengths by stabilizing two different states Pr and Pfr, with specific biochemical activities. However, they show an additional point of regulation: the thermal dark reversion into one of the states. We studied the dark reversion by cryo-trapping a bathy phytochrome at different time points after illumination. This allowed the reconstruction of a PrPfr hybrid state as an intermediate of the previously solved end states, PrPr and PfrPfr respectively. Using cryoSPHERE, we are able to determine the structural heterogeneity from single cryo-EM images of all states by resolving whole protein conformational ensembles. This enables us to highlight the highly dynamic behavior of different regions and proposes an unknown opening mechanism. Additionally, Molecular Dynamics simulations present a detailed inspection of the chromophore binding site coinciding with found asymmetry in cryo-EM maps and suggesting a unique interaction pattern upon dark reversion. Especially the conserved His277 is a key interaction partner for the transitioning from one state to the other. Overall, these results facilitate a more detailed understanding of the dark reversion process on different levels to guide a closer insight into the functioning of phytochromes.

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13. Structural basis for circadian control in the cell

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Earth has a 24-hour cycle during which the temperature and amount of light vary drastically. This daily cycle affects almost all life forms, from single-celled organisms to humans. Circadianvcontrol is an important mechanism in the cell, as it regulates metabolism at different times of the day. The circadian control functions through a negative transcription–translation feedback loop that is regulated by circadian activator and repressor complexes. The activator complex upregulates the expression of the repressor complex proteins, which bind to the activator complex once transported into the nucleus. When the repressor proteins bind to the activator complex, transcription is interrupted. The activator complex binds a specific DNA sequence with high affinity. This binding can be utilized for the purification of the native protein complex from mammalian cell cultures, where the cells have been synchronized to express the same circadian time. Using methods such as DNA affinity chromatography and size-exclusion chromatography, it is possible to retrieve a relatively pure protein complex that has not been modified for the purification process. These truly native protein complexes can offer new insights into the structure and function of the circadian complexes.

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14. Elucidating the Structures of Blue-Light Photoreceptor Proteins and Their Light-Induced Intermediate States

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Phototropins are essential blue-light photoreceptors in plants and algae1. They play a key role in mediating many light-driven functions like chloroplast movement, phototaxis, and phototropism2-4. In the two well-studied organisms, Arabidopsis thaliana and Chlamydomonas reinhardtii, two photosensory LOV (Light, Oxygen, Voltage-sensing) domains are associated with the chromophore FMN (flavin mononucleotide) and regulate a kinase domain5,6. Upon illumination, a covalent bond is formed between the FMN and a conserved cysteine residue, ultimately activating the phototropin kinase domain and translating the light signal into a chemical one.

Despite their important biological roles, no high-resolution structure of full-length phototropins is currently available. To address this, we aim to utilise single-particle cryo-EM to reconstruct the structures of A. thaliana and C. reinhardtii phototropins. Furthermore, we plan to implement time-resolved single-particle cryo-EM to resolve the distinct intermediate states involved in the photocycle.

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15. Time-resolved serial crystallographic study of ultrafast electron transfer reactions in photoreceptor proteins

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The theme of my research is to study electron transfer reactions in proteins using time-resolved X-ray crystallography. I am currently working with photolyase, a light-activated enzyme that repairs UV-damaged DNA. The very first stages of the photoresponse of this protein involves an ultrafast electron transfer step which occurs in less than 500 fs. The goal of my project is to capture time-resolved structural data at an XFEL to see how the protein matrix changes over the course of this electron transfer reaction. Revealing the femtosecond structural changes around this charge transfer reaction will provide a detailed view on the photoactivation mechanism in photolyases and related proteins. Wider impact is expected in that this research will also lay a foundation for understanding chemical charge transfer theory. The reorganization of solvent and the protein matrix itself as charge transfer occurs is one of the most important parameters for understanding the efficiency of e.g. solar energy conversion and catalysis. This research may therefore provide insights towards engineering

proteins with improved electron transport chains, such as in artificial photosynthesis or in DNA repair.

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16. Uncovering structural details behind the [FeFe]-Hydrogenase catalytic cycle

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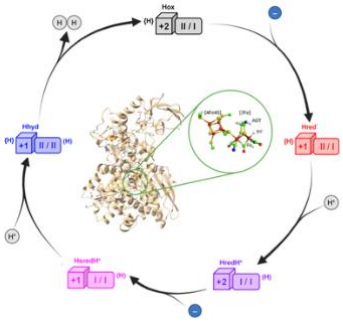

[FeFe]-hydrogenases are metalloenzymes that can catalyse conversion of hydrogen ($2H^+ + 2e^- \leftrightarrow H_2$) with high turnover rate, making them prospective target for hydrogen production that doesn't require fossil fuels.All [FeFe]-hydrogenases possess a unique oxygen-sensitive diiron cofactor that is inactive on its own[1]. The hydrogen production within [FeFe]-hydrogenases occurs at the cofactor through a series of electron transfers between iron atoms and proton binding. The intermediate states of this catalytic cycle have been discovered using various spectroscopic methods[2]. Despite broad spectroscopic data, only structures of ground, oxidised state[3] and CO-inhibited[4] state have been solved using X-ray crystallography, while all the other structures of reaction intermediates remain a mystery. Here, we provide an overview on production and purification of [FeFe]-hydrogenase from *Clostridium pasteurianum*, with current progress on protein microcrystal production. The crystals will be used for *in crystallo* infrared spectroscopy to confirm intermediate redox states of the cofactor and X-ray crystallography for structural studies of the intermediates. The results of this work will provide crucial structural information on currently unavailable intermediate protein states, opening new doors in structure-functional relationship of green hydrogen synthesis.

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17. How is carboxysomal carbonic anhydrase regulated?

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Cyanobacteria utilize light and CO2 for growth and production via the Calvin cycle, with Rubisco as the main but imperfect carboxylase - it also reacts with O2 triggering photorespiration. To boost CO2 fixation, cyanobacteria evolved a CO2 concentration mechanism (CCM), co-encapsulating Rubisco and carbonic anhydrase (CA) into proteinaceous shells called carboxysomes (CBs). HCO_3^- accumulates in the cytosol and enters the CBs. Here

it converts to CO_2 by the CA, increasing local CO_2 levels near Rubisco, which reduces O_2 interference and enhances the overall carboxylation rate. The activity of CA must be tightly controlled to prevent cytosolic HCO_3^- from converting into CO_2 and diffusing out of the cell, disrupting the CCM. It has been shown that at least one carboxysomal CA is redox regulated: inactive in the reducing cytosol and active in the oxidizing interior of CB. Using the stopped-flow based Khalifa/pH indicator assay to measure CA enzyme kinetics, we demonstrate here that another carboxysomal CA also follows this redox pattern. To understand the mechanistic details, a mutation analysis reveals that a conserved cysteine pair is directly involved in the regulation. CryoEM studies of the wild-type and mutant enzyme under varying redox conditions revealed significant local changes close to the active site (see poster Gaullier et al). This study advances our understanding of CA regulation, guiding future efforts to engineer cyanobacteria and plants for enhanced CO_2 fixation.

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18. Structures of active and inactive carboxysomal carbonic anhydrase

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See abstract for short talks.

Tiselius and Unchartered Enzyme Functions

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The separation methods developed in Uppsala by The Svedberg, Arne Tiselius, and their followers have enabled the isolation and structural characterization of macromolecules of biological relevance and medical applications. However, the relationship between structure and function is unknown in many cases, and efficient enzyme catalysis remains particularly difficult to predict. Experimental enzymology thus stays on as a prominent research area. We discovered that one among the numerous glutathione transferases (GSTs) in mosquitoes unexpectedly displays outstanding ketosteroid isomerase activity, comparable to the most efficient of all enzymes known. The enzyme is essential to the growth and survival of mosquitoes, and thus affords a target for combating malaria and other deadly diseases transmitted by the insects. Beyond such naturally occurring enzymes, we are currently designing GSTs for clinical applications in cancer therapy based on epitope-binding fusion proteins and GST-activated prodrugs.