Thursday, September 22, Session I

L1

CRYSTAL STRUCTURES OF COPPER SENSING BACTERIAL HISTIDINE KINASE

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The CusS histidine kinase is a component of the bacterial two-component signal transduction system CusS-CusR, engaged in *Escherichia coli* to prevent the damaging accumulation of copper ions [1]. The CusS, like classical histidine kinase, is a transmembrane multidomain protein [2]. It binds copper by the periplasmic sensor domain and propagates this signal toward the cytoplasmic catalytic core, through the coordinated conformational change of its subsequent domains. Then, the kinase core binds ATP, autophosphorylates its conserved histidine residue and transmits the -phosphoryl group to its cognate response regulator CusR. As a consequence, the response regulator binds to the target operon, responsible for the synthesis of copper-efflux pomp and regulates its transcription [3]. A small amount of copper ions is indispensable for aerobic cell metabolism. Nonetheless, its excess in the cytoplasm generates damaging reactive hydroxyl radicals [4]. For that reason, understanding bacterial copper sensing mechanisms can contribute to reducing bacterial resistance and developing bactericidal copper-based materials.

Using X-ray crystallography, the crystal structure of the CusS kinase core was solved at the resolution of 1.4 Å. The cytoplasmic catalytic domains ensemble in a homodimer structure. The CusS kinase core structure determination allowed us studying intramolecular and intermolecular interactions crucial for the mechanism of CusS autophosphorylation. Based on obtained structural data, conserved catalytic and structural motifs were identified and described. According to identified conserved motifs CusS can be classified into the Type I family of histidine kinases, the most common group of these enzymes constituting 72 % of all histidine kinases.

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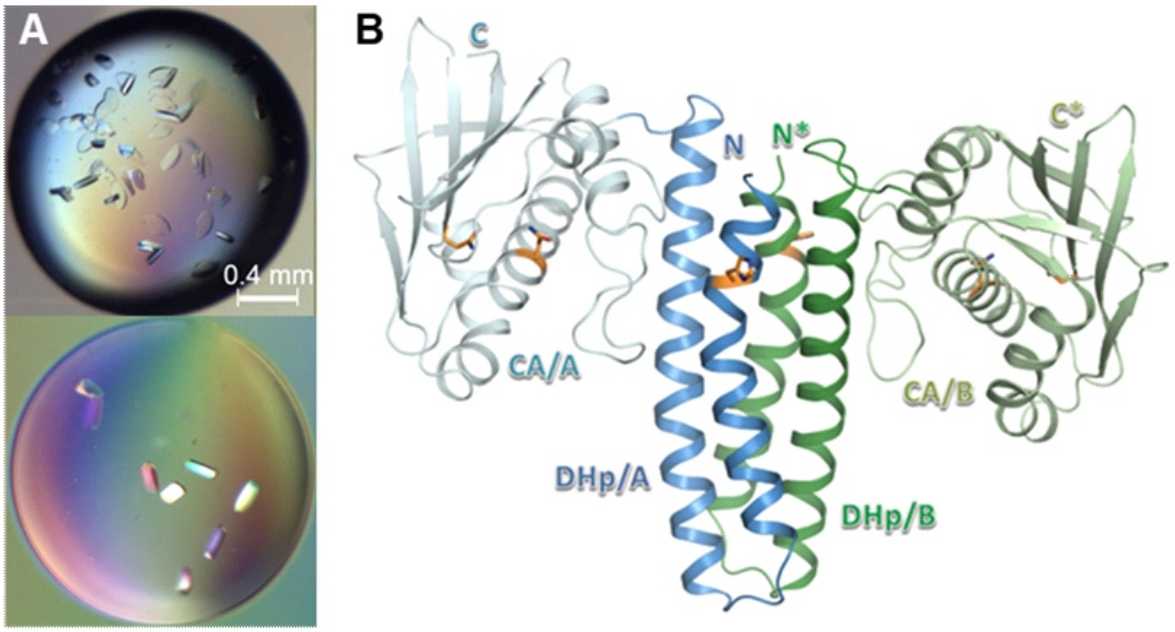

Figure 1: (A) Protein crystals of CusS kinase obtained by hanging drop vapor diffusion technique. (B) The overall structure of CusS catalytic kinase core.

L2

STRUCTURAL AND FUNCTIONAL INVESTIGATIONS INTO GLYCOCIN-GLYCOSYLTRANSFERASES

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Antimicrobial resistance is on the rise. Thus, there is an urgent need to discover novel antimicrobials and determine their mechanism of action.

An underexplored class are bacteriocins: Short ribosomally synthesized and post-translationally modified peptides (RiPPs) that often show high potency, and low toxicity. [1, 2]

One subclass requires a glycosylation to show antimicrobial activity and thus have been termed glycoactive bacteriocins, Glycocins [3].

Their challenging synthesis, *in vitro* or *in vivo*, renders the elucidation of their mechanism and their broader use difficult. Reasonable yields of authentic glycocins could only be obtained by the use of the cognate glycosyltransferase that is able to either glycosylate a serine or threonine, resulting in canonical *O*-glycosylation or a cysteine, resulting in a rare type of *S*-glycosylation. [4-6]

Insights into the function of Glycocin- Glycosyltransferases (GGTs) will help to understand the unusual specificity of these transferases for cysteine and/or serine and threonine and the high selectivity for its glycosylation site. Understanding the key elements of the catalytic site may allow to use the transferases as valuable tools to synthesise glycocins, glycopeptides and neo-glycopeptides in general. We identified several putative Glycocins and their cognate GGTs using bioinformatics. The recombinant production and purification of five selected GGTs from *Bacillus subtilis, Enterococcus faecalis, Gottfriedia acidiceleris, Laceyella sacchari and Streptomyces platensis,* was established.

The GGTs were characterised in regard of the metal ion dependency and the carbonucleotide specificity.

Using SPPS we synthesised some glycocins and proved their selective glycosylation by the cognate GGT. To investigate the molecular determinants for the observed differences in specificity in terms of preferred sugars and their S/O-selectivity, we use X-ray crystallography as method of choice. For three GGTs crystals could be obtained and the structure for the BsGGT could be solved to 2.6 Å resolution.

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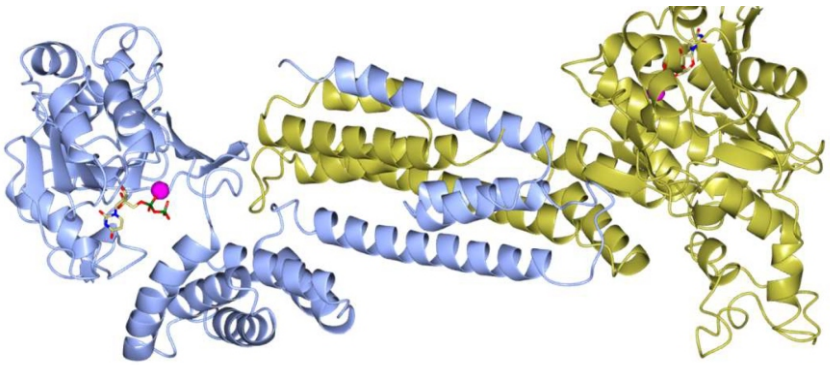

Figure 1. Crystal structure of the crystallographic dimer of B. subtilis GGT at 2.6 Å.

L3

INHIBITION OF A BACTERIAL GLOBAL GENE REGULATOR BY A PHAGE PROTEIN *VIA* FORCED HYPER-OLIGOMERIZATION

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Most bacteria with sequenced genomes encode , a hexameric RNA-dependent ATPase. In many of these species, is essential. is a paradigmatic transcription termination factor that defines the ends of 20-30 % of transcription units in *Escherichia coli*. Based on this capacity, also mediates attenuation in 5'-untranslated regions, limits the extent of antisense transcription, silences foreign genes and safeguards genomes by restricting R-loops. activity involves conformational transitions between an inactive open-spiral and an active closed-ring state, modulated, among others, by nucleotide and RNA binding. On RNA polymerase, -dependent termination can be counteracted by various transcription factors [1], but only two known protein inhibitors target isolated , including the polarity suppression protein, Psu, of pirate bacteriophage P4. Presently, the molecular basis of Psu-mediated ń inhibition is unknown. We elucidated cryogenic electron microscopy structures of -Psu complexes, in which multiple Psu dimers laterally clamp two spirals, stabilizing them in an open conformation. Remarkably, Psu increases the helical pitch of the spirals, fostering their expansion by further ń subunits up to the nonameric state. Consistent with Psu trapping ATP analogs at their binding sites on , ATP stabilizes the -Psu interaction, and Psu reduces ATP binding kinetics, thereby inhibiting the ATPase. Also, fully consistent with the structures, Psu counteracts RNA binding at the center of the spirals, which requires ring closure. Structure-guided exchange of -Psu contact residues reduced complex formation, decreased Psu-mediated ATPase inhibition and undermined Psu-mediated inhibition of -dependent termination *in vivo*. Our findings reveal that Psu implements a unique mechanism – forced hyper-oligomerization – to inhibit , which may inform the development of novel, -targeting anti-microbials.

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L4

HIGH-RESOLUTION STRUCTURE OF HUMAN NUCLEOSIDE DIPHOSPHATE KINASE C IN COMPLEX WITH DIFFERENT NUCLEOTIDES

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Nucleoside diphosphate kinases (NDPK-C) belong to the NM23 protein family and catalyze the reversible phosphorylation of nucleoside diphosphates to nucleoside triphosphates in a magnesium-dependent manner. The enzymes exist as homo- or heteromeric hexamers, the latter are formed with other isoforms. We described a 1.4Å resolution structure of human homo-hexameric NDPK-C bound to ADP. We also analyzed the structure of NDPK-C in the presence of GDP, UDP, and cAMP and described the high-resolution structures of the as-yet unidentified complexes. Both of the partial reactions of the NDPK phosphotransferase activity require Mg^{2+} ions. We provided the structural basis by comparing NDPK-C nucleotide complexes in the presence and absence of this ion. To investigate the nucleotide impact on the NDPK-C conformation, we analyzed a nucleotide-depleted NDPK-C structure and compared it with the nucleotide bound form. The analysis revealed a conformational change upon substrate binding and allowed us to identify flexible regions in the substrate binding site.

L5

HALF WAY TO HYPUSINE. MOLECULAR BASIS OF (DEOXY)HYPUSINATION

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The eukaryotic translation factor 5A (eIF5A) plays a pivotal role during translation. It is the only cellular protein known to undergo hypusination, a unique post-translational modification of a conserved lysine (Lys50 in human eIF5A). Hypusination is essential to resolve ribosomal stalling during the formation of proline-rich polypeptides. Recent findings show that the hypusination of eIF5A plays a role in many important cellular processes, including autophagy, senescence, polyamine homeostasis, and the determination of helper T cell lineages. Malfunctions of the hypusination pathway, including those caused by mutations within the pathway encoding genes, are associated with such conditions as cancer or neurodegeneration. Therefore, hypusination seems as an attractive molecular target for therapeutic interventions.

Hypusination involves two distinct enzymatic steps. First, deoxyhypusine synthase (DHS) catalyzes the transfer of 4-aminobutyl moiety of spermidine to a specific lysine of eIF5A precursor in an NAD-dependent manner. Subsequently deoxyhypusine is further hydroxylated to the mature form hypusine by second enzyme: deoxyhypusine hydroxylase (DOHH).

Here, we present the cryoEM structure of the human eIF5A-DHS complex at 2.8Å resolution and a crystal structure of DHS trapped in the key reaction transition state. Furthermore, using combined structural biology and biochemical analysis, we show that DHS variants that cause neurodegeneration influence complex formation and hypusination efficiency. Hence, our data provide the molecular basis of deoxyhypusine synthesis and reveal how clinically-relevant mutations affect this crucial cellular process.

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L6

HIGH-RESOLUTION STRUCTURE ANALYSIS OF A SMALL PLANT BIOCATALYST AT SUB 2Å RESOLUTION BY CRYO-EM

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More than ever a sustainable and efficient alternative to traditional chemical synthesis based on the deployment of mineral oil is prerequisite in the chemical and pharmaceutical industry. Enzyme catalysis combines both demands, as biocatalysts function under mild reaction conditions and possess excellent selectivity. Particularly plants provide biosynthetic enzymes with exquisite specificity that facilitate complex reactions, like the formation of intricate terpene carbon skeletons. Single particle analysis of the structure of such small proteins has a tremendous potential to increase the rational element of protein engineering and spark the capability of e.g. Cryo-EM for structure solution [1]. Cryo-EM already revealed a stunning success in structure solution of large molecular machines, however a major challenge for this technique are small proteins, such as a large amount of biorelevant catalysts are. Small protein complexes have been only reported to a maximum resolution of 3.0 Å yet [2]. Hence, single particle analysis by Cryo-EM towards the rapid elucidation of small protein structures has a vast potential to increase the rational element of structural biochemistry and protein engineering in the future. Here, we present the highest resolution achieved by Cryo-EM so far. The structure of a ~120 kDa plant borneol dehydrogenase with outstanding volume clearly below 2Å resolution (Figure 1) [3]. Considering that structures of homomultimeric plant enzymes are highly under-represented to date and given the molecular weight of the tetrameric complex, we were able to push the boundaries of this rapidly evolving method drastically. Hence, Cryo-EM is a valuable tool to achieve fast and high-resolution structure determination for enzymes that proved difficult to crystallize.

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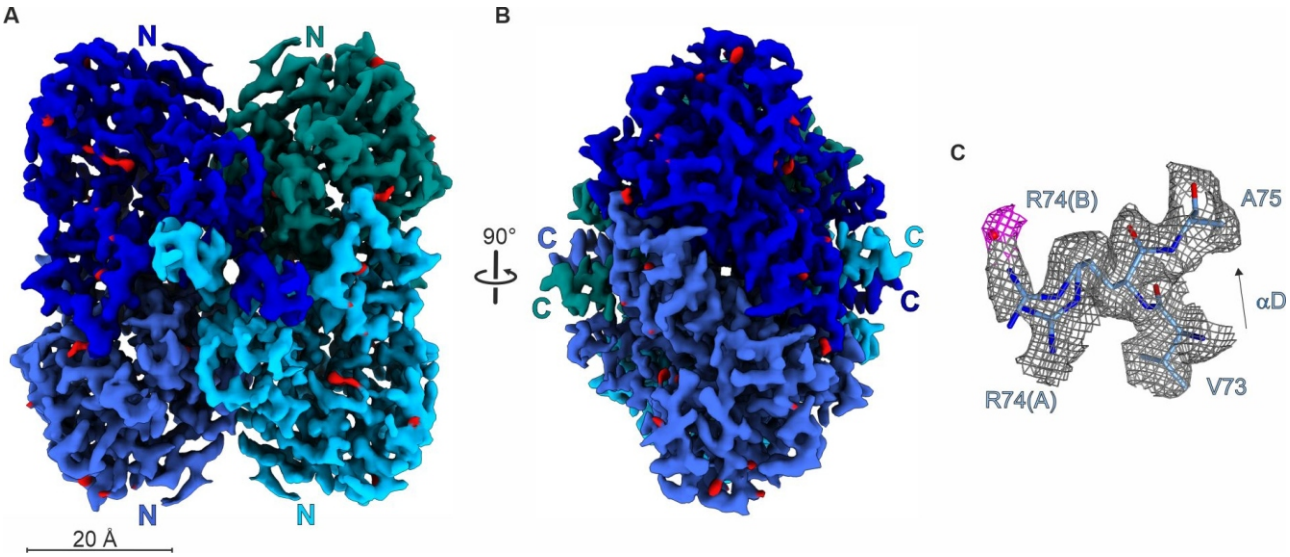

Figure 1: *Sr*BDH1 Cryo-EM structure at 1.88 Å. (A) Tetrameric assembly of *Sr*BDH1. Density of each of the four protomers is shown in different blue tones. (B) Rotation by 90°, same color-coding as in A. (C) Zoom on C-terminal end of H with well-depicted density-hole for aromatic residue F260. (D) Residue R74 in double conformation and water molecule in 2.5 Å distance to guanidinium function. [3]

FT1

FIRST INSIGHTS INTO THE STRUCTURE AND FUNCTION OF THE HUMAN GLYCOGEN DEBRANCHING ENZYME

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Glycogen is the most important short-term reserve of carbon and energy, consisting of -1,4 linked glucose with a regular -1,6 branch every 8-12 residues. Complete degradation of glycogen requires the action of the glycogen phosphorylase and the glycogen debranching enzyme (GDE). Human GDE is a multi-domain enzyme with dual activity, having glucanotransferase and glycosidase activity. First, GDE transfers a maltosyl/maltotriosyl group from a branch to a neighbouring linear chain via its transferase activity. Second, it cleaves the last glucose residue of the remaining branch, thus allowing the glycogen phosphorylase to hydrolyse the linear part of glycogen [1]. GDE mutations can lead to a rare genetic syndrome, characterised by liver, skeletal muscle and/or heart dysfunctions [1, 2]. Available data on structure and function are mainly derived from yeast homologs [2, 3], whereas human GDE (hGDE) is poorly understood on a molecular level.

Here, we show that 175 kDa hGDE expressed in *Escherichia coli* and purified by liquid chromatography is functional and monomeric. We aim to characterise the function of its different domains by rational design of protein constructs, combined with biochemical assays and structural analysis, to shed light on the important functional motifs for efficient catalysis. Our CryoEM structure shows that the overall architecture of hGDE agrees with the previously released crystal structure of a characterised yeast homologue [2]. Additionally, suitable crystallisation conditions were identified by biased hanging-drop crystallisation experiments, paving the way to an integrative structural analysis of this enzyme. Together, the collected results will provide insights into the catalytic mechanism and the overall interaction of the enzyme with its complex, natural substrate. Moreover, the role of disease-causing mutations will be assessed, paving the way to a deeper understanding of the clinical features observed in patients.

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