Friday, September 23, Session IV

L17

E2/E3-INDEPENDENT UBIQUITIN-LIKE PROTEIN CONJUGATION BY URM1 PROTECTS THE PROTEOME UNDER OXIDATIVE STRESS

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Posttranslational modifications by ubiquitin-like proteins (UBL) are essential for nearly all cellular processes. Ubiquitin-related modifier 1 (Urm1) is a non-canonical UBL that plays a key role in tRNA anticodon thiolation as a sulfur carrier protein (SCP). While Urm1 has also been observed to conjugate directly to target proteins like other UBLs, the mechanism of attachment as well as how the SCP properties of Urm1 may impact its conjugation is unknown. Here, we reconstitute the covalent attachment of Urm1 to various cellular target proteins *in vitro*, revealing that, unlike other known UBLs, this process is E2/E3-independent, and conjugates to lysine, serine, threonine residues and requires oxidative stress conditions. We determined the crystal structures of the peroxiredoxin Ahp1 before and after the covalent attachment of Urm1. Strikingly, we show that Urm1 actively transfers sulfur atoms to proteins as part of its conjugation reaction, resulting in the persulfidation of a cysteine residue in the target protein. Our results redefine Urm1 as a key evolutionary link between prokaryotic SCPs and the plethora of UBL modifications in eukaryotes and demonstrate a critical role for Urm1 in protecting proteins during oxidative stress.

L18

IN-SOLUTION STRUCTURE AND OLIGOMERIZATION OF HUMAN HISTONE DEACETYLASE 6 - AN INTEGRATIVE APPROACH

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Histone deacetylases (HADCs) belong to the family of enzymes that remove the acetyl group from lysine side chains of target proteins regulating thus a plethora of cellular process. Among all other HDACs, HDAC6 is a large (140 kDa) and structurally complex multidomain enzyme harbouring a mosaic of unstructured and globular domains (Fig 1). Its primarily found in cytoplasm and acts on many non-histone targets including tubulin, Hsp90, and peroxiredoxins [1-5]. Structural data available currently are only on isolated globular domains and given its structural complexity, the full-length human HDAC6 is a challenging target for X-ray crystallography. To glean

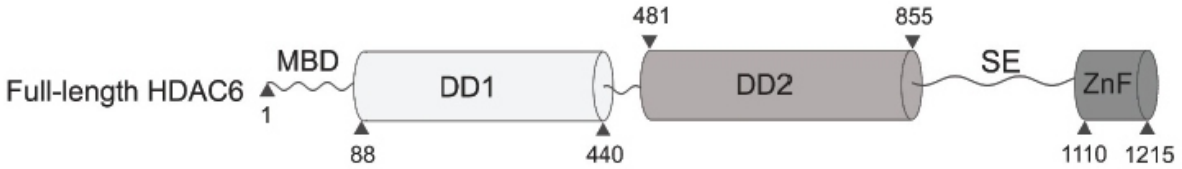

Figure 1. A schematic representation describing the domain organization of full-length HDAC6.

structural information on full-length human HDAC6, we used an integrative approach by combining experimental data from several orthogonal biophysical techniques including analytical ultracentrifugation (AUC), size-exclusion chromatography-multiangle light scattering (SEC-MALS), native mass spectrometry (MS), H/D exchange and small-angle X-ray scattering (SAXS). Our in-solution structural model shows that HDAC6 exists as an ensemble of conformers in solution. Furthermore, our data shed light on HDAC6 concentration-dependent oligomerization mediated by mannerist N-terminal domain. Overall, our findings can be used for further research into structure-function and physiological studies of this unique deacetylase.

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We acknowledge the Grant agency of the Charles University (Project No: 1678218) for their support.

L19

STRUCTURAL STUDIES OF *RHIZOBIUM ETLI* INDUCIBLE ASPARAGINASE MUTANTS

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L-Asparaginases are a large family of enzymes, grouped into three structural Classes. Some Class 1 asparaginases from bacteria are used to treat acute lymphoblastic leukemia (ALL) and lymphosarcoma. Unfortunately, these therapeutic regimens are often associated with a number of serious side effects. Alternative sources of therapeutic asparaginases have thus been sought, and the inducible *Rhizobium etli* enzyme (ReAV) emerges as an interesting candidate.

ReAV differs significantly in sequence from other microbial asparaginases, indicating a different catalytic mechanism of asparagine hydrolysis. The crystal structure of ReAV [1] shows a protein folded as some ß-lactamases, but forming a unique dimeric assembly. The active site of ReAV contains two Ser-Lys tandems, centered around the hydrated Ser48 residue and located in the close vicinity of a Zn^{2+} cation, which has an unusual coordination sphere created by two cysteines, a lysine and a water molecule. The presence of a Zn^{2+} cation in the active site area is unique to ReAV; however the metal ion is not necessary for catalysis. Another characteristic residue of ReAV is an oxidized Cys249, which is involved in a network of H-bonds comprising the active site area.

To decipher the catalytic mechanism of ReAV, the most conspicuous residues implicated by the crystal structure, i.e. the two Ser-Lys tandems (Ser48-Lys51 and Ser80-Lys263), the residues involved in zinc coordination (Cys135, Lys138, Cys189), and the relatively distant Cys249, were subjected to site-directed mutagenesis and substituted with Ala. All eight alanine mutants were studied using biophysical and structural methods. With the exception of the K138A mutant, all the created ReAV muteins lost the ability to hydrolyze L-asparagine, as clearly demonstrated by the Nessler method. This confirms the significance of the implicated residues in catalysis. The replacement of Ser48 and Ser80 by Ala affected the protein stability and folding, as indicated by CD spectra and low expression yields. We were able to crystallize mutants: S48A, K51A, S80A, C135A, K138A, C189A and K263A, and solve their X-ray crystal structures. The structures reveal some intriguing variations in the active site area. With alanine substitutions of Cys135, Lys138 and Cys189, the zinc coordination site fell apart and the mutants are unable to bind Zn. Moreover, the absence of the Zn^{2+} cation affected the oxidation state of Cys249, which no longer carried a chemical modification. The K51A and K263A mutations disrupted the network of H-bonds in the active site region and modified the hydration pattern of Ser48.

Work supported by National Science Centre (NCN, Poland) grant 2020/37/B/NZ1/03250.

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L20

STRUCTURAL ANALYSIS OF METABOLIC BINDING PARTNERS OF SuhB

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Crystallographic fragment screening is a method to obtain insight into ligand binding sites in a protein. These binding events provide a starting point for drug discovery [1]. Ligand binding sites are not only important for drug development, but can also give insight of the function of a protein in the cell. As there are still many proteins with unknown cellular function, ligand or metabolic binding partners can provide a first starting point to deteminate the protein's function.

In this study, a metabolite screen was designed and assembled based on the 26 most abundant metabolites in *Escherichia coli* [2]. Therefore, the metabolites were concentrated to a 10x higher concentration than their intracellular concentration, and dried onto a 96-well plate for soaking expermients. The protein SuhB was chosen to validate the metabolite screen. Validation of the screen revealed four possible metabolic binding parteners for SuhB: 6-phosphogluconic acid, L(-)-malic acid, 3-phosphoglyceric acid and L-glyceric acid. 6-phosphogluconic acid binds to the active site of SuhB and is therefore an interesting target for further enzymatic activity assays. In an isothermal titration calorimetry measurement, the binding affinity of the metabolite to the protein could not be determined, probably due to too low affinity between the ligand and the protein. L-glyceric acid binds in the active site as well were usually a Mg^{2+} ion binds. As 3-phosphoglyceric acid and L(-)-malic acid bind between two symmetry mates in the crystal, it makes these hits rather uninteresting for further analysis.

The validation of the metabolite screen was successful, and the screen might be used in the future for the discovery of metabolic binding partners. 6-phosphogluconic acid might be a new binding partner of SuhB as it binds in the active site. Further analysis and especially validation of this binding event is needed to confirm the results of the screen.

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CRYSTALLOGRAPHIC FRAGMENT SCREEN ON THE C-DI-AMP SYNTHESIZING ENZYME CdaA

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Cyclic-di-AMP is an essential second messenger metabolite which occurs mostly in gram positive bacteria. It regulates various cell processes like the bacterial osmolyte and potassium ion homeostasis. c-di-AMP is produced by enzymes that possess a diadenylate cyclase domain (DAC) and have been identified in several human-pathogen bacteria. In several of them CdaA is the sole and therefor essential enzyme responsible for production of c-di-AMP (1). This renders CdaA a promising target for the development of new antibiotics. As method to identify potential CdaA inhibitors we employed the fragment-based drug design approach relying on crystal structures.

Crystallization of N-terminal truncated CdaAs from different organisms like *E. faecium*, *S. pneumoniae*, *L. monocytogenes* and *B. subtilis* in various catalytic states allow us to gain a deep knowledge about the underlying mechanism of the enzymatic function (2). Furthermore, we were able to obtain structural details at true atomic resolution, enabling the execution of a fragment screening campaign. This approach delivered structural snapshots of multiple compounds bound not only to the active centre of CdaA but also at other regions. These fragments can serve as starting points for the design of follow-up compounds, which can finally lead to a highly specific inhibitor of CdaA.

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L22

THE IMPLEMENTATION OF EXPLICIT WATER MOLECULES IN SIDE CHAIN PACKING ALGORITHMS FOR PROTEIN DESIGN

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Structure-based computational protein design is an effective tool for the *de novo* generation of protein binding pockets and the optimization of protein-ligand interactions. As for all computational models, there is a trade-off between accuracy and computational feasibility. In contrast to detail-oriented methods like molecular dynamics simulations, side chain packing algorithms, as in our inhouse program MUMBO, are based on highly simplified models of physics and conformational flexibility. Here, the solvent is usually considered implicitly, the backbone of the structure is kept mostly rigid and the conformational flexibility is simulated using rotamer libraries [1]. This enables the *in silico* screening of huge mutant libraries with reasonable computational power to optimize protein-protein interfaces, protein-ligand interactions or to stabilize protein folds.

However, when designing protein binding pockets, these simplifications might be inadequate, because essential interactions like stacking or water- and ion-mediated interactions between the protein and the ligand are not taken into account. Since water-mediated interactions often contribute significantly to the affinity and specificity of ligand binding, we have now implemented this type of interaction in the side chain-packing program MUMBO. Explicit water molecules are added between pairs of rotamers at physically ideal positions (Fig. 1), which leads to a considerable reduction in computational complexity compared to the conventional use of solvated rotamers.

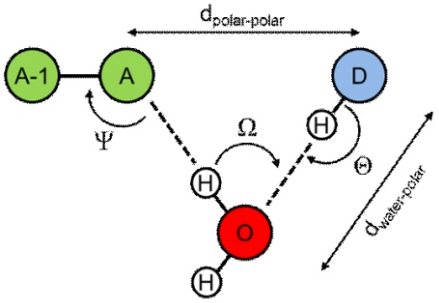

Figure 1. Schematic representation of a water-mediated interaction between an H-bond acceptor and a donor group. The acceptor atom, the acceptor -1 atom (green), the donor atom (blue), the water molecule (red) as well as the for this interaction significant distances and angles are shown.

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CL4

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