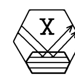

Saturday, September 24, Session VI

L27

NAVIGATING THE STRUCTURAL AND STABILITY LANDSCAPE OF *DE NOVO* TIM BARRELS BY PROTEIN DESIGN AND ENGINEERING

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The ability to create and engineer stable proteins with custom functions is a fundamental goal in biochemistry, with practical relevance for our environment and society. Protein stability can be fine-tuned by modifying diverse structural features such as hydrogen-bond networks, salt bridges, hydrophobic cores, disulfide bonds, loop extension, or protein-protein interfaces, among others. One of the most abundant topologies in nature and a common functional scaffold that is of interest in this context is the ( )$_8$-barrel or TIM-barrel fold [1]. Therefore, we designed and engineered a collection of stable *de novo* TIM barrels (DeNovoTIMs) using a computational fixed-backbone and a modular approach based on improved hydrophobic packing [2] and the introduction of salt-bridges [3]. DeNovoTIMs were subjected to thorough biochemical and folding analyses using computational, biophysical, structural, and thermodynamic methods to explore their structure and stability. We found that DeNovoTIMs navigate a region of the stability landscape previously uncharted by natural proteins, with variations spanning 60 degrees in melting temperature and 22 kcal per mol in conformational stability throughout the designs. Significant non-additive or epistatic effects were observed in their stability and structural features when stabilizing mutations from different barrel regions were combined (Fig. 1). Salt-bridge variants from some DeNovoTIMs exhibit important differences in comparison with the parental proteins, both in conformational stability and structural properties (Fig. 2). The engineering of stable proteins increases the applicability of *de novo* proteins and provides crucial information on the molecular determinants of the sequence-structure-stability relationships, with this study being an essential step towards fine-tuned modulation of protein stability by protein design.

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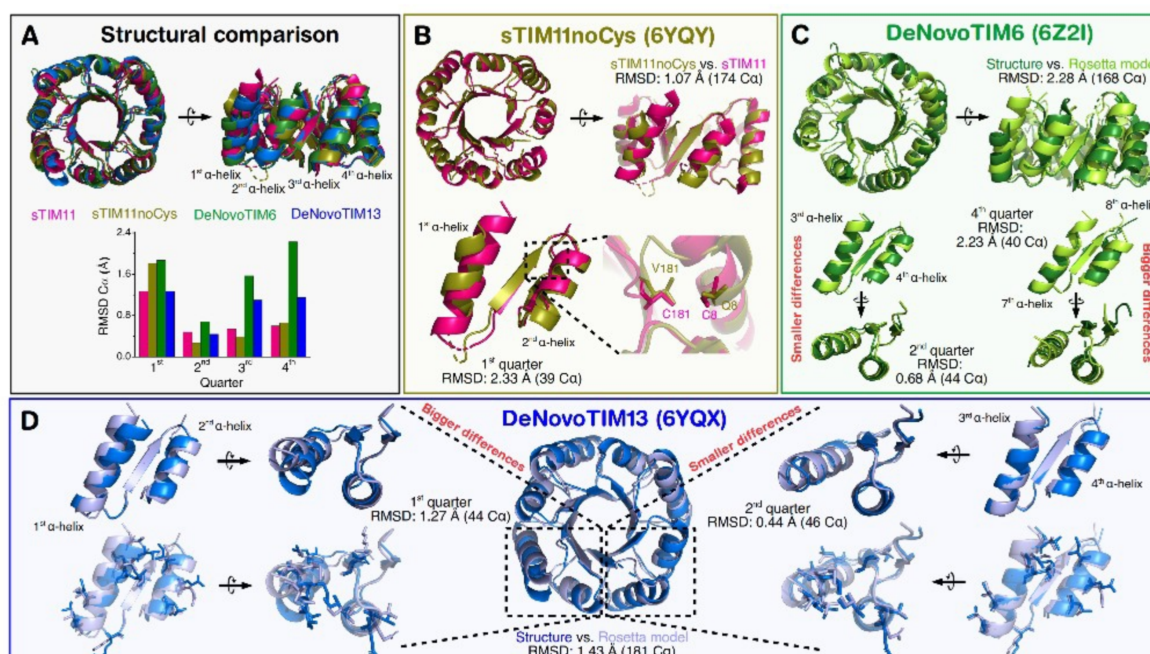

Figure 1. Structural properties observed in the DeNovoTIM collection [2].

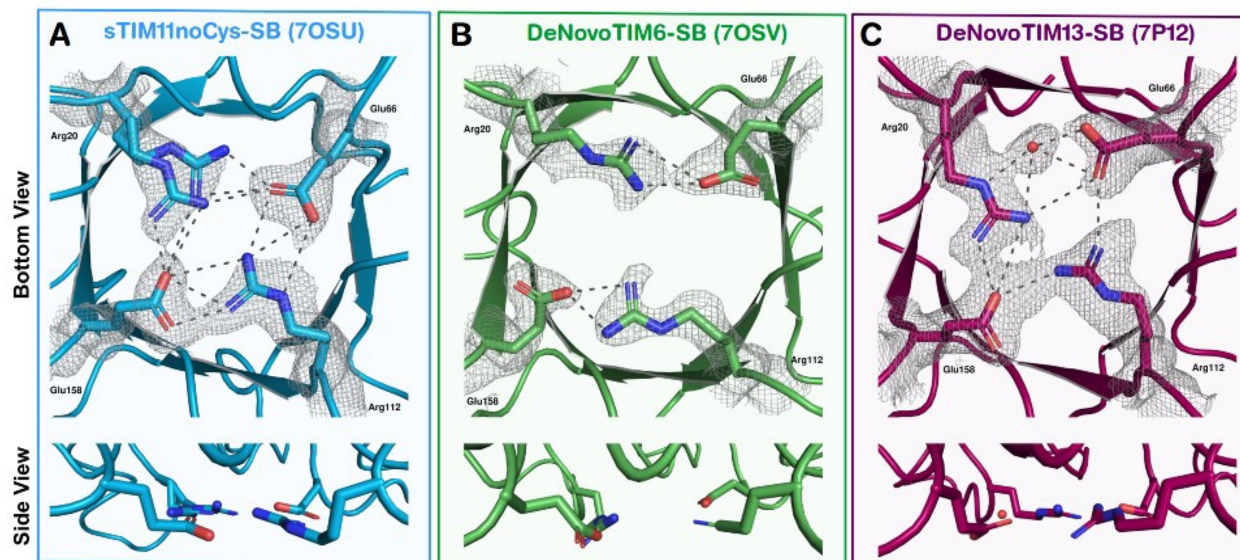

Figure 2. Structural conformations of the salt bridge interactions in the DeNovoTIM-SB variants [3].

L28

INTERFERENCE OF ENVIRONMENTAL COMPOUNDS WITH PPAR

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Obesity is characterized by excessive body fat accumulation and is, therefore, a major risk factor for the development of physical and mental disorders [1]. Humans and other organisms have evolved metabolic pathways which control lipid homeostasis. Among numerous signaling pathways adipogenesis is regulated by peroxisome proliferator-activated receptor gamma (PPAR) cascades which are modulated by ligands of PPAR , such as endogenous polyunsaturated fatty acids but also environmental compounds.

Phthalate di(2-ethylhexyl) phthalate (DEHP) and its substitute cyclohexanoate cyclohexane-1,2-dicarboxylic acid diisononyl ester (DINCH) which are widely used as plasticizers are known for their effect in impaired health [2, 3]. Previous surface plasmon resonance (SPR) data and studies on adipocytes already suggest a direct binding of MEHP to PPAR but sufficient data for MINCH are still missing [4, 5]. Moreover, a detailed structural understanding of how these plasticizers interact with PPAR and, as a consequence, affect human health is lacking until now. To answer this, we structurally characterize the molecular mode by which plasticizers target PPAR and compared their binding to known endogenous, and synthetic agonists of PPAR . Using crystallography, we identified the binding site of MEHP and MINCH in the human PPAR and described the conformational changes of PPAR and its PPAR -RXR interaction surface upon ligand binding, characterizing these compounds as PPAR agonists.

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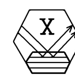

L29

STRUCTURE DETERMINATION OF A PROTEIN-PEPTIDE COMPLEX USING MICROCRYSTAL ELECTRON DIFFRACTION

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Developments in microcrystal electron diffraction (MicroED) allow the determination of protein structures from crystals that are too small to be analyzed by conventional X-ray crystallography [1-3]. Although the method has been used to determine novel protein structures, it is limited to micro/submicron ultra-thin protein crystals [4,5]. Due to the limited rotation range of the sample stage in a MicroED setup, completeness of the collected data is restricted by crystal shape, symmetry and orientation on the grid [4, 6]. Here we applied MicroED to determine the structure of a novel complex between an enzyme regulatory domain and an intrinsically disordered peptide. Needle-like crystals in the space group $P2_12_12_1$ were grown using the hanging drop vapor diffusion method. Diffraction data were collected on a 200 kV Thermo Scientific Glacios Cryo-TEM equipped with a CetaD detector. Data were collected along multiple sections of several crystal needles within the limiting rotation range of the sample stage. The performed collection strategy allowed data processing from only two crystals that were oriented perpendicular to each other on the grid, resulting in a 3.2 Å resolution dataset with a merged completeness of 89.3%. Our work represents an effective workflow for obtaining a complete electron diffraction dataset from needle-like protein crystals.

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L30

ACTIVITY AND STRUCTURE OF HUMAN (D)CTP SPECIFIC DEAMINASE CDADC1

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A balanced dNTP pool is essential for accurate DNA replication, DNA repair, V(D)J recombination, somatic hypermutation, and other cellular processes. Cytidine deamin ation plays an important role in the maintenance of the dNTP pool by bridging the levels of various forms of cytidine/2'-deoxycydinine, 2'-deoxyuridine, and 2'-deoxythymidine [1,2]. In humans, there are three cytidine deaminases that act on free nucleosides/nucleotides. Cytidine deaminase (CDA) deaminates non-phosphorylated forms of cytidine and 2'-deoxycydinine, and is well-studied due to its implication in cancer chemoresistance. Deoxycytidylate deaminase (DCTD) is known to deaminate dCMP yielding dUMP – a substrate for thymidylate synthetase. Surprisingly, very little is known about the third deaminase, cytidine and dCMP deaminase domain-containing protein 1 (CDADC1). Its mRNA and

protein expression profiles suggest a potential role in the immune system and/or in the development of the male reproductive system [3,4]. Unlike CDA and DCTD, CDADC1 is composed of two deaminase domains. Interestingly, the N-terminal domain lacks the otherwise conserved catalytic glutamate residue, suggesting a regulatory rather than catalytic role [5].

In this study, we provide a biochemical and structural characterization of human CDADC1. We found that CDADC1 has preferential and robust activity on CTP and dCTP, which has never been reported in eukaryotes before. The enzyme also deaminates (d)CMP with reduced activity. It is inactive towards cytosine bases and non-phosphorylated nucleosides. Interestingly, CDADC1 has moderate activity on 5-methyl-dCTP, but it's completely inactive on 5-methyl-dCMP. We show that single amino acid substitution in the C-terminal domain (E400A) completely abolishes CDADC1 activity, and therefore, confirm the anticipated catalytic inactivity of the N-terminal domain. We were able to solve Cryo-EM structures of human CDADC1 wild-type at ~4.7Å resolution, and of its catalytically inactive mutant E400A in the presence of dCTP at ~3 Å. The structures revealed that CDADC1 is a homohexamer, arranged as a stack of two trimers. Importantly, the structures show that that the active sites of the C-terminal domains are in close proximity with pseudo-active sites of the N-terminal domains of the adjacent protomers within each trimer. The CDADC1 quaternary structure and its activity pattern support a regulatory/allosteric role of the N-terminal deaminase domain.

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FT16

STRUCTURES OF A DYW DOMAIN SHED FIRST LIGHT ON A UNIQUE PLANT RNA EDITING REGULATION PRINCIPLE

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As part of RNA editosomal protein complexes, pentatricopeptide repeat (PPR) proteins with a C-terminal DYW domain have been characterized as site-specific factors for C to U RNA editing in plant mitochondria and plastids [1-2]. While substrate recognition is conferred by their repetitive PPR tract, the exact role of the DYW domain has not been clarified. The DYW domain shares a low sequence conservation with known cytidine deaminase structures (from 5 to 19% residue identities). Lastly, missing structural information has left the exact function and catalytic properties of DYW domains within the RNA editosome open [3]. We present structures and functional data of a DYW domain in an inactive ground state and a catalytically activated conformation. DYW domains harbour a cytidine deaminase fold and a C-terminal DYW motif, with catalytic and structural Zn atoms, respectively. The deaminase fold is interrupted by a conserved domain, which regulates the active site sterically via a large-scale conformational change and mechanistically via the Zn coordination geometry. Thus, we coined this novel domain 'gating domain' and the accompanying unusual metalloprotein regulation principle 'gated Zn-shutter'. An autoinhibited ground state and its activation by the presence of either ATP, GTP or the inhibitor tetrahydro uridine is consolidated by differential scanning fluorimetry as well as in vivo / vitro RNA editing assays. In vivo, the framework of an active plant RNA editosome triggers the release of DYW autoinhibition to ensure a controlled, coordinated deamination likely playing a key role in mitochondrial and chloroplast homeostasis [4, 5].

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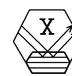

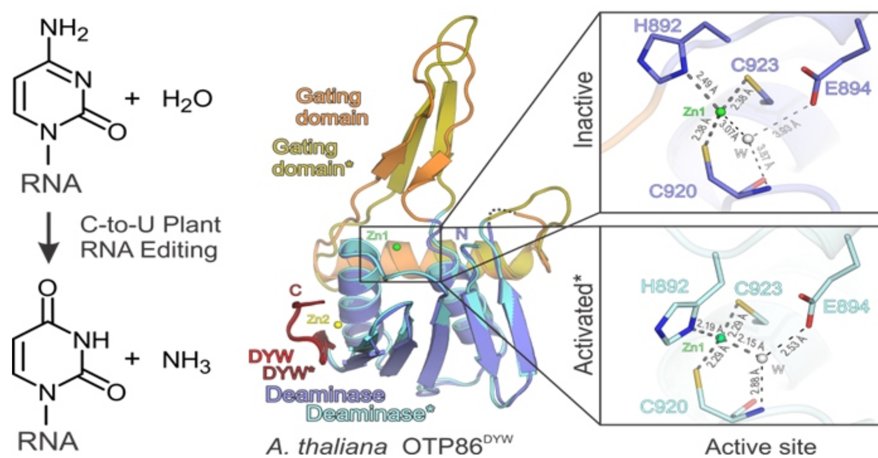

Figure 1. An unusual regulation mechanism in plant RNA

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