Friday, March 25, Session VI

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CAN MISFOLDED ENZYMES BE BENEFICIAL? YES, THEY CAN

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The functionality of a protein catalyst (enzyme) depends on its unique three-dimensional structure, which is a result of the folding process when the nascent polypeptide follows a funnel-like energy landscape to reach a global energy minimum. Computer-encoded algorithms are increasingly employed to stabilize native proteins for use in research and biotechnology applications [1].

Here, we reveal a unique example where the computational stabilization of a monomeric -hydrolase fold enzyme (T_m = 73.5°C; T_m > 23°C) affected the protein folding energy landscape. Introduction of eleven single-point stabilizing mutations based on force field calculations and evolutionary analysis yielded catalytically active domain-swapped intermediates trapped in local energy minima. Crystallographic structures revealed that these stabilizing mutations target cryptic hinge regions and newly introduced secondary interfaces, making extensive non-covalent interactions between the intertwined misfolded protomers [2]. The existence of domain-swapped dimers in a solution is confirmed experimentally by data obtained from SAXS and crosslinking mass spectrometry. Unfolding experiments showed that the domain-swapped dimers could be irreversibly converted into native-like monomers, suggesting that the domain-swapping occurs exclusively *in vivo* [2]. Crucially, the swapped-dimers exhibited advantageous catalytic properties such as an increased catalytic rate and elimination of substrate inhibition. These findings provide additional enzyme engineering avenues for next-generation protein catalysts.

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L30

INTERACTIONS OF ADAPTOR PROTEIN Grb2 WITH MICROTUBULE ASSOCIATED PROTEIN 2c

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MAP2/Tau is a family of neuronal structural microtubule associated proteins (MAPs) known for their ability to bind and stabilize microtubules. Microtubule associated protein 2c (MAP2c) is the shortest isoform of MAP2 that is expressed in neurons of developing brain and brain regions that retain neuronal plasticity in adulthood [1]. Besides binding to tubulin, MAP2c also interacts with proteins involved in signal transduction pathways, such as Grb2 [2], which is an adaptor protein involved in the Ras/Raf/MEK/ERK signaling cascade that is associated with the cell proliferation and apoptosis regulation [3]. Grb2 was reported to bind MAP2c through its SH3 domains [2] but the interaction was not characterized in a residue-specific manner. Therefore, we investigated the interaction of MAP2c with Grb2 using solution NMR. We acquired HNCO spectra to identify residues of MAP2c involved in the interaction with Grb2. The interactions of MAP2c with its binding partners, including Grb2, are regulated both by serine/threonine and tyrosine phosphorylation [2, 4]. Therefore, we employed NMR to investigate the influence

of MAP2c phosphorylation by serine/threonine kinases PKA and ERK2 and tyrosine kinase Fyn on this interaction.

The results will help to elucidate the involvement of MAP2c in the signal transduction pathways and the regulation of its interactions with relevant binding partners by serine/threonine and tyrosine kinases.

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STRUCTURE AND BIOLOGICAL FUNCTIONS OF TBEV CAPSID PROTEIN

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Tick-borne encephalitis virus (TBEV) is the most medically relevant tick-transmitted flavivirus in Eurasia, targeting the host central nervous system and frequently causing severe encephalitis [1]. The primary function of its capsid protein (TBEVC) is to recruit viral RNA and form nucleocapsid [2].

Here we show the first 3D structure of a member of tick-born flavivirus C protein, TBEVC. The structure of monomeric 16-TBEVC was determined using high-resolution multidimensional NMR spectroscopy. Based on natural *in vitro* TBEVC homodimerization, the dimeric- interfaces were identified by hydrogen deuterium exchange mass spectrometry (Fig. 1). Although assembly of flaviviruses takes place in endoplasmic reticulum-derived vesicles [2], we observed that TBEVC protein also accumulated in nuclei and nucleoli of infected cells. Predicted bipartite nuclear localization sequence (bNLS) in the TBEVC C-terminal part was confirmed experimentally.

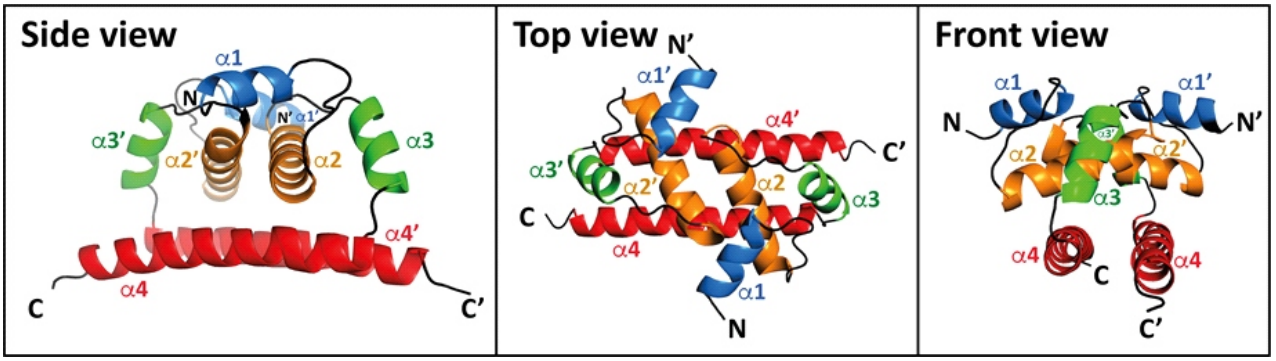

Figure 1: NMR structure of 16-TBEVC homodimer represented as cartoon. The monomeric protein consists of four -helices marked as 1- 4. Generated with the PyMOL program (Schrödinger).

The interface between TBEVC bNLS and import adapter protein importin-alpha was described using X-ray crystallography. Co-immunoprecipitation coupled with mass spectroscopy identification revealed 214 interaction partners of TBEV C including viral E and NS5 proteins and a wide variety of proteins involved mainly in rRNA processing and translational initiation.

Described findings may substantially help to design a targeted therapy against TBEV based on the novel functions of its capsid protein.

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BIOPHYSICAL CHARACTERIZATION OF THE FOXO4:p53 COMPLEX

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Transcription factor p53 protects cells against tumorigenesis when subjected to various cellular stresses [1]. Under these conditions, p53 interacts with transcription factor Forkhead box O (FOXO) 4, thereby inducing cellular senescence by upregulating the transcription of senescence-associated protein p21 [2, 3]. However, the structural details of this interaction remain unclear. Here, we characterize the interaction between p53 and FOXO4 by NMR, chemical cross-linking, and analytical ultracentrifugation. Our results reveal that the interaction between p53 TAD and the FOXO4 Forkhead domain is essential for the overall stability of the p53:FOXO4 complex. Furthermore, contacts involving the N-terminal segment of FOXO4, the C-terminal negative regulatory domain of p53 and the DNA-binding domains of both proteins stabilize the complex, whose formation blocks p53 binding to DNA but without affecting the DNA-binding properties of FOXO4. Therefore, our structural findings may help to understand the intertwined functions of p53 and FOXO4 in cellular homeostasis, longevity, and stress response.

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SEEING THE INVISIBLE – STUDY OF TRANSIENTLY FORMED PROTEIN CONFORMATION FOUND IN DOMAIN 1.1 OF BACTERIAL TRANSCRIPTION FACTOR

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Biomolecules undergo a variety of motions at various timescales. Motions at microsecond to millisecond timescales are often associated with transitions between ground states and higher energy states. Methods of structural biology allow detailed characterization of ground state structure and dynamics. However, the studies of higher energy state conformations are more difficult, because of their low occupancies and short lifetimes. Due to this fact, studies of excited states are often omitted. But nuclear magnetic resonance provides methods to investigate motions associated with these transitions and structures of excited states.

In here, we investigate such conformational exchange, between well-defined ground state and transiently formed excited state which has been detected in domain 1.1 of primary sigma transcription factor from *Bacillus subtillis*. Sigma factor is essential for initiating the process of transcription, a fundamental cellular process. With the use of relaxation dispersion experiments we obtained structural information about orientations of bond vectors and secondary structure propensities within the excited state. Our results suggest that the excited state (populated only about 3% in the solution at 25 °C) has significantly lower propensity to form a stable secondary structure in the regions of helix I and helix III compared to the ground state.

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A UBIQUITOUS DISORDERED PROTEIN INTERACTION MODULE ORCHESTRATES TRANSCRIPTION ELONGATION

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During eukaryotic transcription elongation, RNA polymerase II (RNAP2) is regulated by a chorus of factors. Here, we identified a common binary interaction module consisting of TFIIS N-terminal domains (TNDs) and natively unstructured TND-interacting motifs (TIMs). This module was conserved among the elongation machinery and linked complexes including transcription factor TFIIS, Mediator, super elongation complex, elongin, IWS1, SPT6, PP1-PNUTS phosphatase, H3K36me3 readers, and other factors. Using nuclear magnetic resonance, live-cell microscopy, and mass spectrometry, we revealed the structural basis for these interactions and found that TND-TIM