Friday, March 22, Session IV

L13

PROTEIN-PROTEIN INTERACTION ANALYSIS IN EDUCATION – A MODEL CASE OF CHYMOTRYPSIN INHIBITOR

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Interactions among proteins are essential for any living entity. They are being studied for decades through various approaches. Over the years, many different techniques and instruments were employed in order to describe individual binding parameters, e.g. stoichiometry, equilibrium binding constants, kinetic constants, or enthalpy and entropy of binding. In order to use these techniques efficiently, it is crucial to understand their principles as well as be able to compare their outcomes, advantages and limitations. For this, the basic university lectures are frequently insufficient since they might lack a more practical approach. Here, various forms of more targeted education (workshops, specialized courses, etc.) based on hands-on exercises play their irreplaceable role.

In order to highlight differences and similarities of the techniques, it is highly desirable to use the same interacting system for all experiments. While there are various systems suggested for individual types of analysis (e.g. Ca^{2+}-EDTA for isothermal titration calorimetry [1] or lysozyme-NAG3 for MST [2]), a more universal interacting couple is more rare to be found. In this talk, we would like to demonstrate an application of bovine pancreas -chymotrypsin and soybean trypsin inhibitor in a specialized university course. Both proteins are easy to obtain from natural sources and therefore commercially available. They interact with $K_d \sim 1$ M, being suitable system for analysis through various techniques including isothermal titration calorimetry, microscale thermophoresis, analytical ultracentrifugation [3] or surface-based techniques. The system can be therefore used for multiple methods in parallel, making it a useful tool for their comparison, highlighting the advantages and disadvantages of each approach. It might be also used for other purposes, such as routine instrument check or cross-instrument or laboratory benchmarking.

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3. Ghirlando, R. The analysis of macromolecular interactions by sedimentation equilibrium. Methods 54(1): 145–156 (2011). doi:10.1016/j.ymeth.2010.12.005.

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L14

STRUCTURAL CHARACTERIZATION OF NOVEL BINDING PARTNER OF MYCOBACTERIAL TRANSCRIPTION INITIATION FACTOR SIGMA-A

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IDRs, or Intrinsically Disordered Regions, are segments of proteins that lack a well-defined three-dimensional structure under physiological conditions. Sigma-A (^{A}) protein is a bacterial transcription factor that plays a vital role in the initiation of transcription by association with RNA polymerase (RNAP) driving transcription initiation. ^{A} responds to environmental signals to regulate the expression of genes crucial for bacterial growth, survival, and adaptation. Through an unbiased search for interacting partners of *M. smegmatis* ^{A}, a number of proteins were discovered, with MoaB2 being the most prominent. Molybdenum cofactor biosynthesis protein B (MoaB2) is a member of a universally conserved protein family involved in catalyzing complex oxygen transfer reactions in the Molybdenum incorporation pathway, a trace element essential in bacteria. Recent studies have shown MoaB2 is catalytically inactive structural homolog in prokaryotes. In this study, we explored several functional experiments which demonstrated that MoaB2 inhibits ^{A}-dependent transcription and increases the biological stability of ^{A}. Several approaches were used to validate and characterize the ^{A}-MoaB2 interaction which demonstrated that the binding was specific and independent of RNAP, as other sigma factors did not show binding to MoaB2. The structural analysis of MoaB2 revealed that the Intrinsically disordered N-terminal domain of ^{A} plays a role in the ^{A}A-MoaB2 interaction. In summary, this study uncovers MoaB2 as a novel binding partner of mycobacterial ^{A}, which suggests it may have adopted an alternate function in prokaryotes.

L15

THE ROLE OF CAROTENOID EXCITED STATE IN ORANGE CAROTENOID PROTEIN ACTIVATION

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The Orange Carotenoid Protein (OCP) is a unique type of protein naturally present in cyanobacteria, serving a photoprotection function. Normally, at daily sunlight levels, the protein remains in its inactive, so-called orange form. However, under extensive photon flux, the protein activates into its “red” form, enabling it to non-photochemically quench excessive energy flowing from the phycobilisome to the reaction center. The photoactivation process exhibits a very low quantum yield (1%), with activation finely tuned for adaptation to fluctuating light levels. This ensures the protein remains inactive under ideal light conditions while effectively managing excessive energy in light-harvesting complexes [1–3].

Another aspect that distinguishes the OCP is the inclusion of carotenoids, such as echinenone, hydroxyechinenone, and canthaxanthin, within its core, where they play a crucial role in light absorption and subsequent protein activation. The carotenoids' strong absorbance of blue-green light is exploited as a "sensor" for light conditions. The absorbed energy can be converted into a form of mechanical motion as needed, activating the protein.

There is an ongoing debate regarding the sequence of events following light absorption that leads to the formation of the red form of OCP, with a consensus that the excited state manifold of the carotenoid plays a vital, initiating role [4–7]. This study aims to elucidate the differences in energy flux between specific excited states of the carotenoid echinenone in various solvents (methanol, acetonitrile, cyclohexane) and when incorporated into the OCP protein.

The investigation utilized Femtosecond Stimulated Raman Spectroscopy (FSRS) in both upshifted and downshifted regions, along with Transient Absorption Spectroscopy. This approach offered a higher level of correlation between vibrational and absorption spectroscopy, enhancing the understanding of the excited state dynamics.

Our findings reveal unique vibrational characteristics of echinenone associated with OCP's photoactivation stages (S2 state). We also identified a notable absence of vibrational signature for echinenone's relaxed S1 state within OCP and observed stronger signals from a highly excited ground state (GS) in OCP. Additionally, the presence of a short-lived intramolecular charge transfer state (ICT) was detected.

These observations are attributed to the altered conformation of carotenoid once embedded in the protein environment.

The study also puts forward a hypothesis regarding the photoactivation mechanism of the Orange Carotenoid Protein (OCP), highlighting the significant role of an extraordinarily high level of excitation in longitudinal stretching modes as the primary driving force. This suggests that the specific vibrational energy states of carotenoids, influenced by their interaction with the protein environment, are crucial for initiating the photoactivation process that leads to the protective red form of OCP.

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L16

MULTIPLE RETINAL ISOMERIZATIONS DURING THE EARLY PHASE OF THE BESTRHODOPSIN PHOTOREACTION

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Bestrhodopsins constitute a class of light-regulated pentameric ion channels that consist of one or two rhodopsins in tandem fused with bestrophin ion channel domains. Here, we report on the isomerization dynamics in the rhodopsin tandem domains of *Phaeocystis antarctica* bestrhodopsin, which binds all-trans retinal via a Schiff-base (RSB) absorbing at 661 nm and, upon illumination, converts to the metastable P540 state with an unusual 11-*cis* RSB isomer. The primary photoproduct P682 corresponds to a mixture of highly distorted 11-*cis* and 13-*cis* RSB isomers directly formed from the excited state in 1.4 ps. P673 evolves from P682 in 500 ps and contains exclusively highly distorted 13-*cis* retinal, indicating that the 11-*cis* fraction in P682 has converted to 13-*cis* RSB. Next, P673 establishes an equilibrium with P595 in 1.2 s, during which RSB converts to 11-*cis* in the latter species and then further proceeds to P560 in 48 s and P540 in 0.8 ms while remaining 11-*cis*. Hence, extensive isomeric switching occurs on the early ground state potential energy surface (PES) on the hundreds of ps to s timescale before finally settling on a metastable 11-*cis* photoproduct. We propose that the early photoproducts P682 and P673 are trapped high up on the ground-state PES after passing through either of two closely located conical intersections that result in 11-*cis* and 13-*cis* RSB. Co-rotation of C11=C12 and C13=C14 bonds results in a constricted conformational landscape that allows thermal switching between 11-*cis* and 13-*cis* species of highly strained RSB chromophores. Protein relaxation may release RSB strain, allowing it to evolve to a stable 11-*cis* isomeric configuration within microseconds.

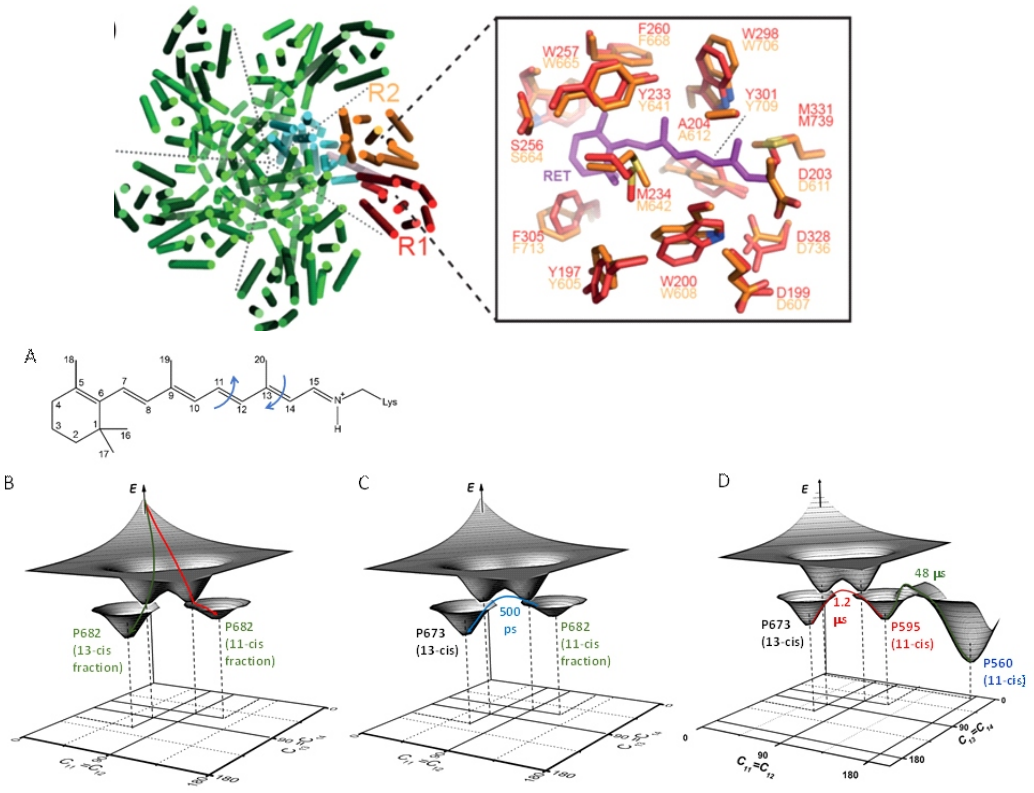

Figure 1. Bestrhodopsin channel structure and potential energy surface of retinal isomerization

Friday, March 22, Session V

L17

A MULTIFACETED ROLE OF FILAMENTOUS HEMAGGLUTININ (FHA) IN THE VIRULENCE OF PATHOGENIC *BORDETELLA* SPECIES

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Filamentous hemagglutinin (FHA), a major virulence factor of classical *Bordetella*e, is a rod-shaped molecule that plays an important role in the adherence of bacteria to ciliated epithelial cells of the upper respiratory tract and suppresses the host innate and adaptive immune response. FHA is translated as a 360-kDa FhaB precursor that is exported across the outer bacterial membrane by a two-partner secretion mechanism involving the outer membrane protein FhaC and shed into external environment as an N-terminal 'mature' 220-kDa FHA protein after processing by surface-exposed SphB1 protease. The remaining C-terminal 130-kDa FhaB prodomain is thought to regulate maturation process and rapidly degraded in the periplasm. We show here that both the extreme C terminus (ECT) of the FhaB prodomain and the mature FHA play the pivotal roles in the virulence of *B. pertussis*. The NMR-based structural analysis of ECT, a highly-conserved the C-terminal 100 residues of the FhaB precursor, revealed that the ECT polypeptide adopts a rigid structure with a 'pilin-like' protein fold. Deletion of the sequence encoding ECT (ECT) resulted in a significant decrease in bacterial colonization within the nasal cavity of infected mice, comparable to *B. pertussis* strain lacking the FhaB precursor (FhaB). Intriguingly, the ECT strain exhibited a complete loss of its ability to bind cilia on human nasal epithelial cells grown at the air-liquid interface, emphasizing the indispensable role of ECT in the adherence of Bordetella cells to ciliated epithelial cells. Furthermore, we demonstrate the mature FHA confers resistance of *B. pertussis* to complement-mediated killing, highlighting its involvement in protection of bacterial cells against the host's innate immune response. Collectively, these results provide novel insights into FHA biology, unraveling its multifaceted role in the virulence of pathogenic *Bordetellae*.

L18

STRUCTURAL BIOLOGY OF ACUTE MYELOID LEUKEMIA (AML) PROTEIN AND SMALL MOLECULE INHIBITOR COMPLEX

Mohanraj Gopalswamy, Manuel Etzkorn, Holger Gohlke

Nanotemper

Acute myeloid leukemia (AML) is a malignant disease of immature myeloid cells and the most prevalent acute leukemia among adults. The oncogenic homo-tetrameric fusion protein RUNX1/ETO results from the chromosomal translocation t(8;21) and is found in AML patients. The nervy homology region 2 (NHR2) domain of ETO mediates tetramerization; this oligomerization is essential for oncogenic activity. Previously, we identified the first-in-class small-molecule inhibitor of NHR2 tetramer formation, **7.44**, which was shown to specifically interfere with NHR2, restore gene expression down-regulated by RUNX1/ETO, inhibit the proliferation of RUNX1/ETO-depending SKNO-1 cells, and reduce the RUNX1/ETO-related tumor growth in a mouse model. However, no biophysical and structural characterization of **7.44** binding to the NHR2 domain has been reported. Likewise, the compound has not been characterized as to physicochemical, pharmacokinetic, and toxicological properties. Here, we characterize the interaction between the NHR2 domain of RUNX1/ETO and **7.44** by biophysical assays and show that **7.44** interferes with NHR2 tetramer stability and leads to an increase in the dimer population of NHR2. The affinity of **7.44** with respect to binding to NHR2 is K_{lig} = 3.75 1.22 M. By NMR spectroscopy combined with molecular dynamics simulations, we show that **7.44** binds with both heteroaromatic moieties to NHR2 and interacts with or leads to conformational changes in the N-termini of the NHR2 tetramer. Finally, we demonstrate that **7.44** has favorable physicochemical, pharmacokinetic, and toxicological properties. Together with biochemical, cellular, and in vivo assessments, the results reveal **7.44** as a lead for further optimization towards targeted therapy of t(8;21) AML.