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Thursday, March 20, Session II

L2

SEQUENCE-BASED POLYMORPHISM OF Tau PROTEIN AMYLOID FIBRILS

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Thanks to advancements in cryo-electron microscopy, the structure and sequence composition of tau amyloid fibrils from different diseases are well-established [1]. However, the exact mechanisms underlying disease-specific mechanisms, spreading patterns, and resulting disease-specific fibrils morphology remain elusive, hindering the development of specific anti-tau therapies. Previously validated amyloid motifs in the tau protein sequence are regarded as carriers of tau amyloidogenicity because of their intrinsic beta propensity and ability to assemble into stable amyloid fibrils core [2-3]. Together with environmental factors and posttranslational modifications (PTMs), these short amyloid-nucleating motifs contribute to the formation of different protofilaments interface, which underpin the amyloid fibrils polymorphism. One of the most common PTMs that plays a key role in the pathogenesis of Alzheimer's disease is truncation, which promotes the self- assembly of tau monomers by exposing regions that are prone to aggregation in Alzheimer's disease [4]. In some cases, however, truncations in amyloid-prone regions can impair further amyloid aggregation. In our study, five variants of different lengths of tau involving different amyloid motifs were used, including two experimentally tested PHF6(306-311) and PAM4(350-362) and predicted G(326-331) and L(341-449) to demonstrate the sequence-dependent aggregation mechanism. All tau variants were truncated at residue 391 from the C-terminus and at the different sites from N-terminal part of the protein (297-391, 306-391, 316-391, 321-391, 326-391). Propensity toward amyloid aggregation of five tau variants was evaluated by ThT fluorescence assay, and the presence of polymorphic amyloid fibrils was confirmed by atomic force microscopy under four aggregation conditions – with or without heparin and DTT. Tau321-391 exhibits the highest amyloid robustness across all tested conditions, being the only tau variant to produce filaments under physiological conditions represented by pure PBS buffer. On the other hand, tau326-391 variant failed to produce the fibrils even in the presence of heparin, indicating the crucial role of 321-325 for tau monomers self-assembly.

Molecular dynamics simulations revealed an increased propensity of 321-325 sequence toward beta-structures in all atom simulations but a rather helical propensity in coarse-grained simulations. Tau321-391 monomers interacted through a helical interface, which was already featured for other amyloid proteins in early stages of aggregation. MD simulations successfully replicated sequence-specific beta-sheet propensity in agreement with computationally predicted and experimentally established amyloid-nucleating motifs.

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L3

RNA POLYMERASE SUBUNIT DELTA - ELUSIVE PLAYER IN BACTERIAL TRANSCRIPTION

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Since the 80s, the -subunit is in the spotlight of many researchers who tried to solve the mystery of its function. It was first described in *Bacillus subtilis*. However, it became apparent that it is part of the transcription machinery in many *Firmicutes*, like *Staphylococcus aureus* or *Streptococcus pyogenes*. It is also present in intracellular bacterial parasites from class *Mollicutes*. -subunit is associated with the bacterial RNA polymerase (binding partner of its subunit '), where it plays a crucial role in transcription initiation and termination. It was demonstrated that it serves a role of major transcription regulator of genes, that are responsible for environmental adaptation, virulence and sporulation [1,2,3,4].

Structural studies showed that the -subunit is roughly 20 kDa large protein, consisting of 2 domains. N-terminal globular domain which binds to the RNA polymerase core, and highly acidic and intrinsically disordered C-terminal one. While binding of the N-terminal domain to the RNA polymerase was more or less established, the structure and binding of the C-terminal domain was solved relatively recently by H. H. Pei [3,4,5]. However, current findings shed light only on the transcription termination and recycling of the RNA polymerase complex, while the mechanism and function of the C-terminal domain remain obscured.

In the past, our group structurally characterized the -subunit of *B. subtilis*, and identified an important sequential and structural feature - the lysine tract. We investigated and confirmed, that the negatively charged C-terminal domain interacts with the tract, creating a more compacted structure of the whole protein [6]. In this study, we aimed to describe the relation between the length of the C-terminal domain and the effect of the lysine tract on the transcription and the structure of the -subunit. To probe the possible effects, we compared it to its homolog from *S. aureus*, which naturally lacks the lysine tract. Furthermore, we characterized the *S. aureus* -subunit structure using SAXS and NMR, which gave us some important clues about the nature and function of this regulatory protein.

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L4

PHOTOACTIVATION OF HUMAN GREEN CONE OPSIN STUDIED BY STIMULATED RAMAN SPECTROSCOPY: INITIAL STEPS TOWARD UNDERSTANDING THE EARLY EVENTS IN HUMAN VISION

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Retinal proteins have been the focus of scientific investigation for several decades, leading to a substantial body of knowledge about these pivotal components of the biosphere. However, despite this progress, detailed insights into the dynamics of opsins underlying human vision remain remarkably limited [1]. This knowledge gap arises not from a lack of interest but from significant challenges inherent in the study of these proteins. Firstly, the eukaryotic nature of human opsins renders their preparation highly complex. Secondly, their monostable nature causes the proteins to undergo irreversible disintegration following a single photon absorption. A collaborative effort between biochemists from the Paul Scherrer Institute and metrologists from the ELI Beamlines facility has successfully addressed these challenges. Employing approximately 50 microliters of a human green opsin sample, the researchers captured the photoisomerization process with a high signal-to-noise ratio. This achievement represents the first-ever recorded dynamics of human cone opsins. These findings constitute a significant advancement in our understanding of human vision and provide a foundation for comparative studies with other retinal-driven photobiological processes. Current results suggest surprisingly large differences in the structure and dynamics of cone opsins responsible for color vision compared to the somewhat better-understood rhodopsins that mediate achromatic night vision.

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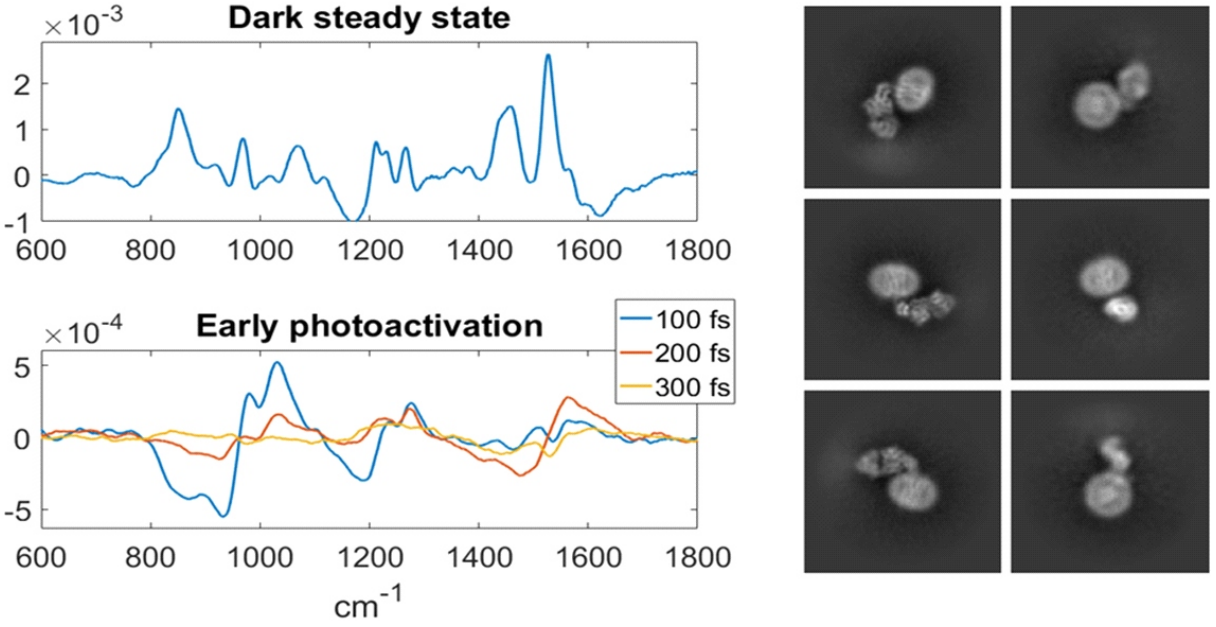

Figure 1. Steady state, time resolved femtosecond stimulated Raman spectra and cryo-EM images of green opsin. Time resolved dynamic was after photoactivation at 560 nm. Results suggests that isomerization is almost fully completed after 200 fs making it even faster than in bovine rhodopsin and arguably the fastest biochemical reaction ever observed.

L5

DEVELOPMENT AND APPLICATION OF CATALYTIC DNA SENSORS

Martin Volek, Jaroslav Kurfürst, Kateřina Švehlová, Matúš Drexler, Michal Svoboda, Martin Jakubec, Milan Kožíšek, Pavel Srb, Václav Veverka, Edward Curtis

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People typically think of DNA as a molecule that stores genetic information, but this remarkable polymer can also have many other functions. These include the ability to catalyze chemical reactions. In this presentation I will talk about catalytic DNA molecules recently developed in our group that generate chemiluminescent, fluorescent, and colorimetric signals. I will also discuss our efforts to convert these DNA enzymes into sensors that only generate a signal in the presence of specific target molecules. Such sensors have great potential for applications such as diagnostics and high-throughput screening.

L6

CHLAMYDIA EFFECTOR CT622/TaiP COMPLEX WITH AUTOPHAGY MASTER REGULATOR ATG16L1

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Chlamydia trachomatis is an obligate intracellular bacterium that relies on a diverse arsenal of secreted effector proteins to manipulate host cellular processes and establish the inclusion, a specialized compartment essential for its proliferation. Among these effectors, CT622/TaiP has emerged as a key regulator of host-pathogen interactions, yet its precise molecular function remains elusive.

Previous findings indicated that CT622/TaiP directly interacts with the WD40 domain of ATG16L1[1], a crucial autophagy regulator involved in autophagosome formation and multiple protein interactions. As part of our collaborative project with Prof. Agathe Subtil (Institut Pasteur), we aimed to characterize this complex and propose a potential

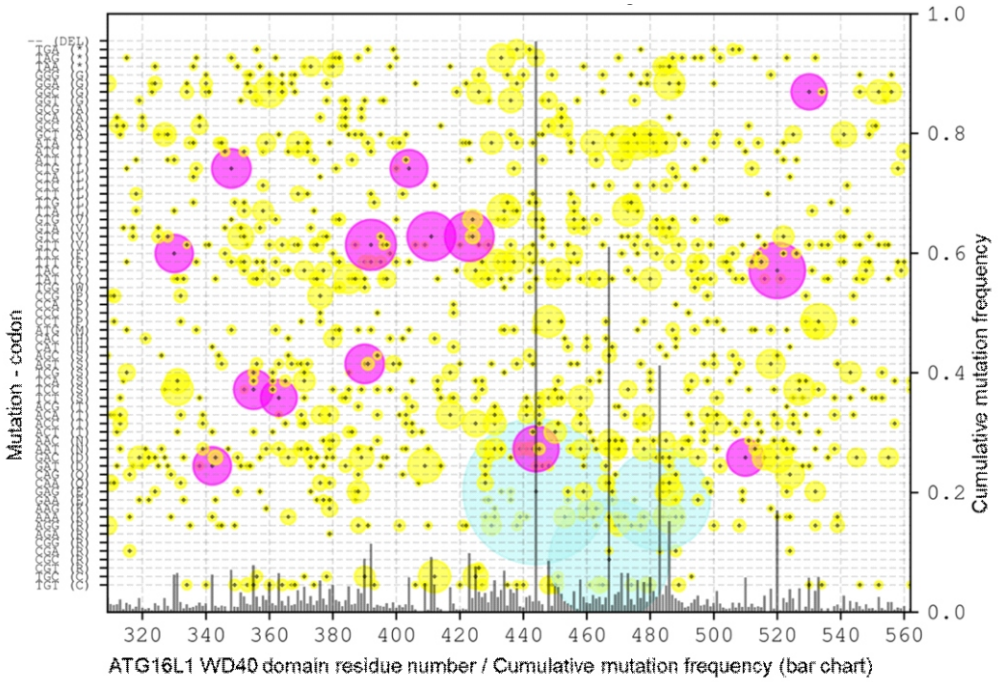

Figure 1. Results of yeast display-based mapping of the CT622/TaiP–ATG16L1 binding site and affinity maturation. Circle size represents mutation frequency within the selected population. Color code: sky blue for mutations with frequency >0.2, magenta for mutations with frequency >0.05 and yellow for mutations with frequency bellow 0.05

mechanism by which *Chlamydia* modulates autophagy to evade degradation.

Determining the experimental structure of this complex presented a significant challenge due to the unfavorable biochemical properties of both proteins, necessitating extensive protein engineering to enhance their stability and affinity. Since predictive algorithms such as AlphaFold-Multimer failed to accurately model the interaction, we employed yeast display-based mapping of the binding site and affinity maturation (Figure 1). To support our mutational data, we utilized hydrogen–deuterium exchange mass spectrometry and mutational analyses, which allowed us to design protein variants sufficiently stable for cryo-EM structure determination.

Our findings provide new insights into the molecular basis of CT622/TaiP–ATG16L1 binding and establish a framework for understanding how *Chlamydia* manipulates the autophagy machinery, with potential implications for therapeutic intervention.

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L7

BEYOND MONOLITH X- THE PRINCIPLES OF SPECTRAL SHIFT

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Among many existing fluorescence-based applications, dedicated to characterization of molecular interactions, vast majority depend on site-specific labeling, binding- induced change of conformation, or size of interacting molecules. To overcome these limitations, we applied a ratio-m etric dual-emission approach that quantifies ligand-induced spectral shifts with sub-nanometer sensitivity. The use of environment-sensitive near-infrared dyes with the method we describe, enables affinity measurements and thermodynamic characterization without the explicit need for site-specific labeling or ligand-induced conformation changes. The newest isothermal spectral shift technology, implemented together with TRIC (temperature related intensity change), in newest NanoTemper system, Monolith X allows researchers to work in solution with variety of biomolecules, including proteins, antibodies, and nucleic acids, as well as with the most challenging types of targets, like membrane or intrinsically disordered proteins.

L8

USE OF FIDA FOR RAPID CHARACTERIZATION OF LIQUID-LIQUID PHASE SEPARATION

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Liquid-Liquid Phase Separation (LLPS) is a phenomenon caused by the spontaneous and reversible formation of condensates that results in a highly concentrated dense phase and a dilute phase [1].

In some cases, liquid to solid transitions occur, causing the formation of amyloid fibrils, amorphous aggregation, and gelation. Even though sometimes beneficial to the cell, these events are mostly associated with detrimental effects related to various neurological disorders such as ALS, Alzheimer's and Parkinson's disease [5]. As a result, LLPS has gained increased attention in academic and industrial settings [6]. Despite this, the field is lacking easily approachable methods for rapid characterization of the key parameters.

This Application Note is based on the paper of Stender, Ray & Norrild et al. published in Nature Communication [3]. It describes how FIDA is used as the new method to rapidly characterise multiple crucial LLPS parameters using L of sample with no need of prior expertise in the technology.

Using Fida 1 as the single experimental platform, we measured dilute phase concentrations, droplet count, relative droplet size distribution, kinetics of droplet formation, maturation into amyloid fibrils as well as the affinity between proteins undergoing LLPS and LLPS-modulating compounds.

More specifically, we analysed the influence of ssDNA on the condensation of the n1 domain of human DEAD-box helicase 4 (Ddx4n1). Ddx4n1 is a protein involved in creating the nuage in egg and sperm cells and is well known for its role in partitioning polynucle-otides [7]. We also present how FIDA is used to study the liquid to solid transition of -synuclein into Thioflavin T positive amyloid fibrils - a process involved in Parkinson's disease-

showing the great potential of the technology for the study of LLPS-related neurological disorders [8].

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