XVIII Discussions in Structural Molecular Biology and 5th User Meeting of the Czech Infrastructure for Integrative Structural Biology

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The event is organized by the Czech Society for Structural Biology, the Czech Infrastructure for Integrative Structural Biology, and the Institute of Biotechnology of the Czech Academy of Sciences.

Thursday, March 24, Session I

L1

TAU GENERATES PROTECTIVE ENVELOPES ON MICROTUBULES

Zdeněk Lánský

Institute of Biotechnology, Czech Academy of Sciences, BIOCEV

Tau is an intrinsically disordered microtubule-binding protein involved in a number of neurodegenerative diseases. Malfunction of tau and its detachment from axonal microtubules is correlated with axonal degeneration. However, mechanistic understanding of this process is still missing. Employing in vitro reconstitution approach combined with single molecule imaging, we recently showed that tau molecules cooperatively form cohesive, selectively permeable envelopes on the microtubule surface. We found that the formation of tau envelopes is governed by structural changes in the microtubule lattice. Importantly, these tau envelopes can protect microtubules from microtubule-degrading enzymes. I will discuss the mechanism of the tau envelope formation and the effect of established neurodegenerative factors on the envelope structure and stability.

L2

STRUCTURAL CHANGES OF CAROTENOID ECHINENONE IN ORANGE CAROTENOID PROTEIN STUDIED BY FEMTOSECOND RAMAN SPECTROSCOPY

M. Kloz[1], P. Čubáková[1], T. Friedrich[2] , T.Polivka[3], E. Maksimov[4]

[1]ELI-Beamlines, Institute of Physics, Praha, Czech Republic
[2]Technische Universität Berlin, Institute of Chemistry PC14, Berlin, Germany
[3]Institute of Physics, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
[3]Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia
miroslav.kloz@eli-beams.eu

The orange carotenoid protein (OCP) [1] shown in the figure 1 is a perfect system to study changes in cofactor structure during photoswitching of proteins by Raman techniques. It hosts a single xanthophyll molecule and undergoes well-studied (but not yet fully understood) photocycle that is associated with the loss of vibrational structure in the absorption spectra.

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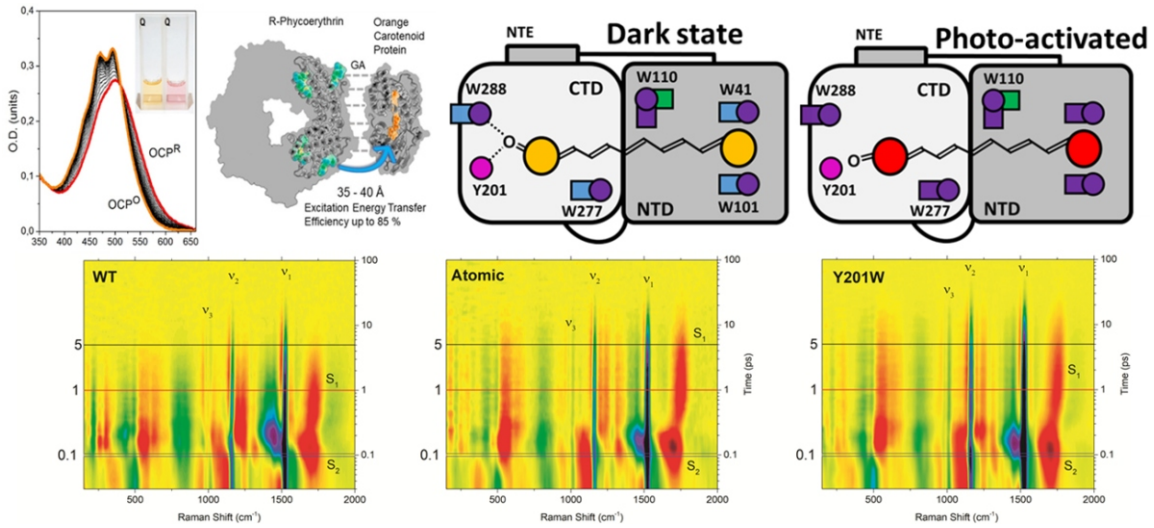

Figure 1. The orange carotenoid protein consists of two subunits that get mutually loose after carotenoid excitation that switches it between the so-called "red" and "orange" states. In that form, it binds to other light-harvesting proteins while greatly increasing their non-radiative decay of excitons. Both the mechanism of OCP photoswitching and its subsequent role as a trigger of non-photochemical quenching is yet to be understood. We studied the wild type and two types of mutants (including utilization of non-canonical amino acids) to understand the role of hydrogen bond formation in the photoswitching mechanism by Stimulated Raman scattering. *(figure is with courtesy of Eugen Maksimov)*

L3

Nedd4-2 BINDING TO 14-3-3 MODULATES THE ACCESSIBILITY OF ITS CATALYTIC SITE AND WW DOMAINS

R. Joshi[1, 2], T. Obsil[1, 2], V. Obsilova[1]

[1] Department of Structural Biology of Signaling Proteins, Division BIOCEV, Institute of Physiology of the Czech Academy of Sciences, 252 50 Vestec, Czech Republic
[2] Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, 12843, Prague, Czech Republic
rohit.joshi@fgu.cas.cz

Neural precursor cells expressed developmentally downregulated protein 4-2 (Nedd4-2), a homologous to the E6-AP Carboxyl Terminus (HECT) ubiquitin ligase, triggers the endocytosis and degradation of its downstream target molecules by regulating signal transduction through interactions with other targets, including 14-3-3 proteins. In

our previous study, we found that 14- 3-3 binding induces a structural rearrangement of Nedd4-2 by inhibiting interactions between its structured domains. Here, we used time-resolved fluorescence intensity and anisotropy decay measurements together with fluorescence quenching and mass spectrometry to further characterize interactions between Nedd4-2 and 14-3-3 proteins. The results showed that 14-3-3 binding affects the emission properties of AEDANS-labelled WW3, WW4 and, to a lesser extent, WW2 domains and reduces their mobility, but not those of the WW1 domain, which remains mobile. In contrast, 14-3-3 binding has the opposite effect on the active site of the HECT domain, which is more solvent exposed and mobile in the complexed form than in the apo-form of Nedd4-2. Overall, our results suggest that steric hindrance of the WW3 and WW4 domains combined with conformational changes in the catalytic domain may account for the 14-3-3 binding-mediated regulation of Nedd4-2.

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L4

PHOSPHOMIMICKING MUTATIONS PHOSPHORYLATION – A CASE STUDY OF 14-3-3 PROTEIN

A. Kozeleková[1,2], A. Náplavová[1], T. Brom[2], Z. Trošanová[1,2], P. Louša[1,2], J. Hritz[1,3]

[1]Central European Institute of Technology, Masaryk University, Kamenice 5, Brno, 625 00, Czechia
[2]National Centre for Biomolecular Research, Masaryk University, Kamenice 5, Brno, 625 00, Czechia
[3]Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, Brno, 625 00, Czechia
aneta.kozelekova@ceitec.muni.cz, jozef.hritz@ceitec.muni.cz

Protein phosphorylation is one of the most common posttranslational modifications that affects protein structure, interactions, or localization. To study the effect of phosphorylation on protein properties, a fully and specifically phosphorylated sample is usually required, although not always achievable. Therefore, phosphorylation is often replaced by phosphomimicking mutation, i. e. mutation of phosphorylatable Ser/Thr/Tyr by negatively charged Asp or Glu [1]. However, how reliable is this approximation of phosphorylation?

In this study, we have focused on dimeric 14-3-3 proteins, regulatory hubs interacting with hundreds of phosphorylated partners [2]. Phosphorylation of 14-3-3 protein at Ser58 has been proposed to induce 14-3-3 monomerization and changes in protein function [3, 4]. However, difficulties with preparation of the phosphorylated sample often led to the usage of phosphomimicking and monomeric mutants [5, 6].

Here, we have prepared the 14-3-3 protein fully and specifically phosphorylated at Ser58 and we have compared its properties with the phosphomimicking mutants (S58D, S58E), frequently used in the literature. We have revealed significant differences in protein oligomeric state, thermal stability, and hydrophobicity [4, 7]. For instance, we have observed disparity in the dimerization dissociation constants of four orders of magnitude. For this reason, we encourage proper verification of protein properties before employment of phosphomimicking mutants.

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L5

NEUTRONS FOR STRUCTURAL BIOLOGY AT THE INSTITUT LAUE LANGEVIN

Mark Johnson

Institut Laue Langevin, Grenoble, France

The ILL is a European research facility, in which the Czech Republic is a prominent member, along with neighbouring countries like Slovakia and Austria. The ILL reactor provides the most intense, continuous neutron beams in the world, in particular for low energy neutrons that are used to study the structure and dynamics of macromolecular complexes. A major, 60 M€, facility upgrade programme is underway in which all of the instruments for structural biology are being significantly improved. In particular, a second protein crystallography instrument has recently been commissioned and the small angle scattering instruments are benefitting from larger detectors, which increase count rate and dynamic range. In this way, significant new capability is being created for structural biology, supported by services for sample preparation (crystal growth, selective deuteration of proteins and natural lipids, etc) and flexible beam time adapted to the needs of the biology community. While neutrons are not the most widely used analytical probe in this field, they do offer unique insight through the sensitivity to light atoms (in particular hydrogen) and contrast variation possibilities by hydrogen-deuterium substitution.

In this talk, I will present an overview of new developments at ILL and illustrate their application in structural biology with recent examples, including, of course, a range of COVID-related experiments.

NEUTRON DIFFRACTION FOR DECIPHERING LECTIN-CARBOHYDRATE INTERACTIONS IN BACTERIAL INFECTION

L. Gajdos[1], M. Blakeley[1], M. Haertlein[1], T. Forsyth[2], J. Devos[1], A. Imberty[3]

[1]Institut Laue-Langevin, 71 Avenue des Martyrs, 38000 Grenoble, France
[2]Faculty of Medicine, Lund University, BMC Biomedical Centre , LINXS Institute for Advanced Neutron and X-ray Science, IDEON Building: Delta 5, Scheelvagen 19, 223 70 Lund, Sweden
[3]Université Grenoble Alpes, CNRS, CERMAV, 38000 Grenoble, France
gajdosl@ill.fr

Lectins are carbohydrate-binding proteins that play important roles in cell recognition and host-pathogen interactions. Many pathogenic bacteria produce lectins that are specific for glycans present on the host surface and that participate in adhesion at the early stages of infection. Lectin-carbohydrate interactions are mostly formed by hydrogen bonds between the sugar hydroxyl groups and the amino acid residues of the lectin binding sites. Other types of interactions can also be involved including CH- stacking, hydrophobic interactions, water-bridging or metal coordination.

Neutron macromolecular crystallography (NMX) offers unique insights into the hydrogen-bonding network as it directly locates and visualizes all hydrogen (or deuterium) atoms. Perdeuteration where all hydrogen atoms are replaced by deuterium atoms enhances their visibility in the neutron maps. While perdeuteration of recombinant proteins is almost routinely carried out in dedicated facilities, the production of perdeuterated sugars is still very challenging.

Using NMX, we have unravelled the details of protein-carbohydrate interactions in two fucose-specific lectins, with the unique feature of producing perdeuterated monosaccharide fucose using a glyco-engineered strain of *E. coli* bacteria for preparation of co-crystals [1]. PLL lectin from bacteria *Photorhabdus luminescens* was chosen as a model system for a detailed description of the H-bonding network involved in sugar recognition, including direct and water-bridged hydrogen bonds and CD-stacking interactions between the apolar face of fucose and aromatic amino acids [2, 3].

LecB lectin from *Pseudomonas aeruginosa,* a human opportunistic pathogen that causes lethal infections in cystic fibrosis patients, is currently viewed as a potential drug target for glycomimetic compounds with antiadhesive properties. LecB displays an unusually high affinity towards fucose with two calcium ions involved in the binding. The neutron study enabled a complete description of the hydrogen-bonding network and the protonation states of charged amino acids involved in the sugar binding including the observation of a low-barrier hydrogen bond between fucose and the protein [4]. The new structural data may help in the design of new potent glycomimetic antiadhesive compounds for fighting antibiotic-resistant bacteria.

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