Friday, March 18, morning, Session III

L10

SECOND GENERATION OF CARBORANE-BASED INHIBITORS OF CARBONIC ANHYDRASES

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Carbonic anhydrases (CAs) play important roles in many physiological and pathophysiological processes. For example, extracellular CAs participate in tumor growth and progression. CAIX, which is selectively expressed in a range of hypoxic tumors, is a validated diagnostic and therapeutic target (recently reviewed in [1–3]). There are 15 human CA isoenzymes, and due to the ubiquity of these enzymes in human tissues, selective inhibition is a very important aspect of drug design.

Mechanism of the inhibition was elucidated for paternal compounds by solving crystal structure of the CA-IX like in complex with these inhibitors. The CA-IX-like enzyme has the active site analogous to CA-IX thanking to site directed mutagenesis of CAII and thus behaves as CAII, it means that crystallizes very well. These X-ray structures proved specific binding of the cluster compounds in the active site of CA-IX and they revealed the key interactions, which are responsible for binding and inhibition at molecular level. Crystal structures were solved for CA-IX-like enzyme in complex with all essential types of carborane and (metalla)carborane inhibitors with both, sulphamide and sulphonamide functional group, several of them with atomic resolution. The structural information has been is consecutively exploited for design of structurally optimized generations of inhibitors.

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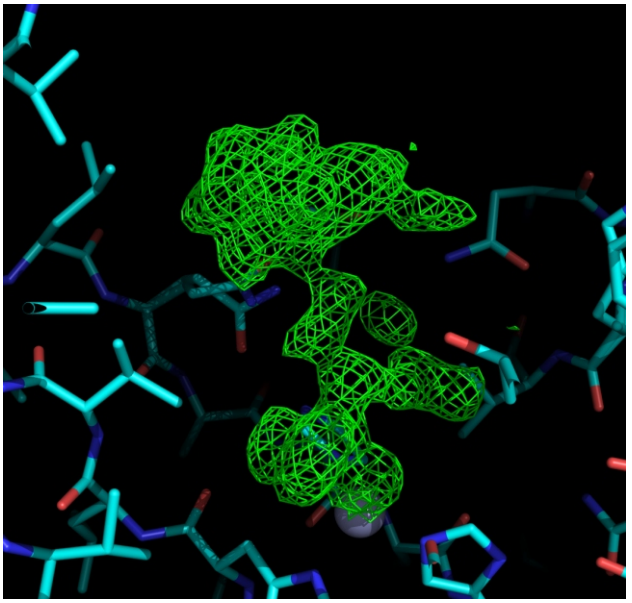

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L11

X-RAY STRUCTURE OF BILIRUBIN OXIDASE FROM *MYROTHECIUM VERRUCARIA* WITH LIGAND IN OXIDATION CENTER

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Blue multicopper oxidases (MCOs) including bilirubin oxidase are enzymes catalyzing oxidation of a substrate accompanied by four electron reduction of molecular oxygen to two molecules of water. Electrons taken from the substrate are transported from the oxidation center, containing copper ion as an electron acceptor, through a highly conserved motive – cysteine-histidine bridge to a reduction center, where they are utilized for oxygen reduction [1].

Bilirubin oxidase from plant pathogen *Myrothecium verrucaria* (*Mv*BO, EC 1.3.3.5) catalyzes oxidation of a great variety of compounds (e.g. bilirubin to biliverdin) with the use of oxygen as a second substrate, where no hydrogen peroxide comes out of the reaction. Therefore, it is interesting for many industrial applications such as pulp bleaching, delignification or development of biosensors and experimental biofuel technology [2].

Although the complete crystal structure of *Mv*BO has been already published (PDB code: 2XLL [3], 3ABG [4]), the mechanism of substrate binding and the way of the electron transport from substrate to the oxidation center still need explanation. On the basis of our latest results, we are able to suggest possible ways of electron transfer from the substrate to the oxidation center.

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L12

THE CONFORMATION OF PROLINE RICH SEGMENT OF NEURONAL PROTEIN TAU STUDIED BY THE X-RAY CRYSTALLOGRAPHY, MOLECULAR DYNAMICS SIMULATIONS AND BIOPHYSICAL METHODS

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Tau is an intrinsically disordered protein (IDP) implicated in Alzheimer's disease. Under physiological conditions, tau associates with microtubules and regulates their dynamics, whereas during the progression of neurodegeneration tau dissociates from microtubules, misfolds and creates deposits in the brain tissue. To unravel the conformational properties of microtubule binding tau sequence located in its proline rich region ^{214}Ser-^{231}Thr the monoclonal antibody Tau5 with epitope in this region was used as a tau protein surrogate binding partner. We have determined the conformation of 16 amino acid tau peptide bound in antibody combining site. The stability of this X-ray observed conformation was probed by molecular dynamics simulation. The simulation results were compared with the results obtained for a peptide with mutation T220A that has shown decreased affinity for Tau5 antibody in biophysical measurements.

To this end we have performed unrestrained MD simulations with 9 amino acid long tau peptide ^{218}PPTREPKKV^{226} that contains all residues that are in contact with antibody paratope in the X-ray structure of the complex, for both wild type and mutated peptide.

This work was supported by the Slovak Research and Development Agency under the contract No. LPP-0038-09 and by VEGA grant 2/0177/15.

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L13

ACTIVE SITE PROMISCUITY IN THE S1-P1 NUCLEASE FAMILY

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Members of the S1-P1 nuclease family (Pfam PF02265) play important roles (e.g. scavenging of nutrients, specific apoptotic functions, and pathogen/symbiont – host interactions) in fungi, plants, single cell eukaryotes and some bacteria. Usually they are -helical proteins with a trinuclear Zn^{2+} cluster in the center of the surface groove. The fold is stabilized by two or more disulfide bridges. Several oligosaccharides bonded on the surface serve primarily as a shielding of the hydrophobic regions and therefore contribute to solubility and stability. These enzymes act as phosphodiesterases cleaving the bond between phosphorus and the 3' hydroxyl group. Hydrolysis of the phosphodiester bond is performed by a nucleophilic attack of the activated water (hydroxide) molecule followed by creation of a penta-coordinated transition state and its breakup into the products. Members of this family are either bifunctional (cleave both single stranded and double stranded forms of DNA and RNA) or single strand-specific. All the studied members of this family have also the 3'-nucleotidase activity.

The active site of the S1-P1 nuclease family members is composed of two distinctive parts. The catalytic zinc cluster is responsible for binding of the phosphate moieties. An adjacent pocket serves as a nucleobase binding site. Ligand binding is also affected by the amino acids in close proximity of the active site. Even though structures of three members of the S1-P1 nuclease family are known [1, 2, 3], these structures contain only few ligands in the active site. Our study of a fungal nuclease significantly broadens our understanding of the ligand binding properties of this family and shows the active site flexibility and existence of yet unexpected binding modes.

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L14

IDENTIFICATION OF THE RH421-BINDING SITE ON Na^+/K^+-ATPase

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Na^+/K^+-ATPase (sodium pump) plays a privileged role in the metabolism of all animal cells. It maintains the resting value of plasma membrane potential and creates a gradient of sodium ions that is essential for function of numerous secondary active transporters. Consequently, inhibition of this enzyme can result in various diseases, such as hypertension, renal failure or diabetes, to name just few.

Styrylpyrimidium dye RH421 (N-(4-sulphobutyl)-4-(4-(p-dipentylaminophenyl)-butadienyl)-pyrimidium inner salt) became one of the most important tools for monitoring of Na^+/K^+-ATPase kinetic. It has been used in several laboratories for characterization of ion binding and translocation of ions across the membrane. The RH421 dye responds to changes of electric charge within the membrane dielectric and it is able to detect charge movements associated with cation binding and dissociation at the extracellular and cytoplasmic surfaces of the protein. It was found that this probe specifically responded to the translocation of the third sodium ion by Na^+/K^+-ATPase. However, structural details of interaction between RH421 and Na^+/K^+-ATPase are still unclear.

We used six single-tryptophan mutants to identify the binding site in FRET experiments. They revealed that RH421 binds directly into the ATP-binding site. This conclusion was further supported by results from molecular docking and by competitive experiments using ATP. Experiments with protein/DPPC mixture revealed that RH421 can bind to both protein and lipids, but only the former interaction was influenced by the presence of ATP.

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Friday, March 18, morning, Session IV

L15

AFFINITY. STABILITY. CONFORMATION.

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MicroScale Thermophoresis (MST) is a powerful technique to quantify biomolecular interactions. It is based on thermophoresis, the directed movement of molecules in a temperature gradient, which strongly depends on a variety of molecular properties such as size, charge, hydration shell or conformation. Thus, this technique is highly sensitive to virtually any change in molecular properties, allowing for a precise quantification of molecular events independent of the size or nature of the investigated specimen. When performing a MST experiment, a temperature gradient is induced by an infrared laser. The directed movement of molecules through the temperature gradient is detected and quantified using either covalently attached or intrinsic fluorophores. By combining the precision of fluorescence detection with the variability and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to dissect molecular interactions.

NanoDSF is our advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan present in virtually all proteins. The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields an ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required as in conventional DSF, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 250 mg/ml down to 5 μg/ml. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control.

The surface acoustic wave technology (SAW) allows for an in-depth analysis of molecular interactions in real time. Binding kinetics can be precisely determined by detecting mass and binding-induced conformational changes. In addition to standard interactions, viscous, colored and turbid samples can be analyzed, and also complex samples including membrane preparations can be investigated. The SAW technology measures changes in mass and conformation separately, thus providing new insights to mechanisms of binding in addition to the binding kinetics (kon, koff and the dissociation constant Kd) and stoichiometry. SAW is based on the precise detection of the properties of surface acoustic waves that travel along the biosensor. Upon interaction with molecules on the sensor surface, distinct characteristics of the acoustic waves are altered; changes in total mass on the biosensor result in a shift of the wave's phase providing information about the on- and off-rates, as well as the stoichiometry of the interaction. Simultaneously a change in flexibility of the molecules alters the wave's amplitude. This directly reflects changes in the conformation of the molecules, e.g. after binding to compounds.Both signal types are detected and quantified separately, and can be used to comprehensively characterize the interaction mechanism of the molecules on a kinetic and structural level.