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Conference Committee

Bohdan Schneider, Ivana Kutá Smatanová, Radomír Kužel, Dalibor Štys, Rudiger Ettrich, Jindřich Hašek

Thursday, March 18, Open forum - future of structural biology in Europe

L1

INSTRUCT – AN INTEGRATED STRUCTURAL BIOLOGY INFRASTRUCTURE FOR EUROPE

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New rules and priorities regarding support of key research infrastructures in Europe are being defined by the European Strategy Forum on Research Infrastructures (ESFRI). Integrated Structural Biology Infrastructure for Europe (INSTRUCT) has been voicing the needs and priorities of the field and will likely play an important role for the future of molecular and cellular structural biology research in Europe in the coming years.

The Czech Republic has joined the effort and become an affiliated country of INSTRUCT in 2008. Our participation in the preparatory phase of INSTRUCT, now extended till March 2011, offers a limited space to influence the development including future policies such as support of access to heavy technology, e.g. synchrotron radiation sources, cutting edge NMR or EM technologies. The project also represents an opportunity for development of some techniques and methodologies (computational approaches and standardization of electron microscopy image processing and other).

The Czech structural biology community is characterized by a wide spectrum of experimental and computational approaches but limited capacity and "sub-critical mass" in many techniques. Formation of a national structural biology platform to foster the future development in this field and represent the needs and benefits of this research field in communication with the Czech Ministry of Education, Youth and Sports and INSTRUCT has been agreed on at the first National User Group meeting in Prague in December 2009.

The function, formal framework and legal status of such national platform are to be discussed and established at this meeting.

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Lectures - Thursday, March 18, Session I

L2

SOLID-STATE NMR OF BIOLOGICAL SAMPLES

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Solid-state nuclear magnetic resonance (NMR) is an invaluable spectroscopic method undergoing rapid development during the past years. In the area of biological research, it focuses on determination of atomic-resolution structures for so-called insoluble proteins, including difficult cases such as membrane proteins, amyloid fibrils, and proteins in heterogenous environments. Through efficient exploitation of isotropic as well as anisotropic (i.e., orientation dependent) nuclear spin interactions, it is now possible to routinely obtain 2- and 3-dimensional solid-state NMR spectra which in many respects resemble those known from liquid-state protein NMR spectroscopy. Such achievements were possible thanks to both improved theoretical understanding and technological advances. The solid-state NMR methodology includes fast and ultra-fast spinning of samples oriented at the magic angle with respect to the static magnetic field (magic angle spinning, MAS), use of strong radio-frequency fields and sophisticated pulse schemes as well as advanced isotope labeling with NMR active nuclei. Using such tools enables to fine-tune the effects of nuclear spin interactions during the course of an experiment and extract relevant structural informations. Beside the necessary assignment of resonances, interatomic distance constraints (or even precise distances) can be extracted and used for structure determination. With an attempt of reaching higher sensitivity, improved efficiencies of magnetization transfers and better spectral resolution, hundreds of different pulse methods have been developed. A general trend is that the highest quality has been obtained using increasingly advanced NMR pulse sequence design principles, including high-order average Hamiltonian theory, numerical optimizations based on analytical models, and most recently using optimal control procedures. All methods have their strong and weak points and much more techniques are expected to appear in this lively area of research.

In order to demonstrate the great potential of the solid-state NMR approach to structural characterization of biological materials, selected examples from the field-leading groups will be presented and major challenges together with some emerging methods will be briefly discussed.

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L3

DETAILED DYNAMICAL ANALYSIS OF WT M-PMV MATRIX PROTEIN AND ITS SINGLE POINT MUTANT R55F WITH RESPECT TO THEIR DIFFERENT OLIGOMERIZATION PROPERTIES

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Mason-Pfizer monkey virus (M-PMV) belongs to the genus of betaretroviruses, in which the matrix protein (MA) plays the essential role in certain stages of their life cycle (e.g. in the assembly, transport and budding of new viral particles). Several single or double point mutants are known to cause dramatic changes in the virus life cycle. In particular, the single point mutation R55F in MA redirects the assembly of the viral capsid to the plasma membrane instead of to cytoplasm, which is the place of assembly of the wild type form (WT).

In our recent work [1] we found that M-PMV WT MA exists in a monomer–dimer–trimer equilibrium in solution with the corresponding dissociation constants of 2.3 mM and 0.24 mM, respectively. Conversely, almost negligible and nonspecific oligomerization was observed for the R55F mutant. Structural comparison of matrix proteins explained their different behavior in solution, concluding that the key residues involved in the intermonomeric interaction are exposed in the WT MA while being buried in the mutant, which prevents the oligomerization of R55F MA.

Here, we present the motional analyses of the experimental ^{15}N NMR relaxation and "in silico" molecular dynamic simulations (MD) for WT and R55F MAs. Both approaches enlighten molecular motions from the microscopic point of view. Analysis of experimental data provides information about global reorientation of molecule, flexibility of each residue (through order parameters, S^2) and for most sites also the timescale of local motion. Although MD simulations provide a wealth of information giving almost complete picture of protein dynamics, careful analysis of results is necessary to avoid possible artifacts coming from poor sampling of rare dynamical processes during simulated trajectory. Two computational approaches were used here; the first one was the isotropical reorientational eigenmode dynamics (iRED) [2] which provided especially information about correlated motions. The second one was the calculation of the timescale window-dependent order parameters, $S^2(w)$, which provided information inaccessible by experiment. Computational routine is implemented in program PAIN [3]. Important changes in local dynamics caused by the single point mutation will be demonstrated and discussed.

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L4

INTERACTION OF THE M-PMV MATRIX PROTEIN WITH PHOSPHATIDYLINOSITOL BISPHOSPHATE

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Polyprotein Gag as a precursor of structural proteins plays a key role in formation and budding of retroviral particles. The N-terminal domain of Gag, the matrix protein (MA) interacts with the cytoplasmic membrane of infected cell through the bipartite signal that involves a cluster of basic residues and myristic acid which is covalently attached to the amino-terminal glycine. In unbound state the myristate is seqestered in MA hydrophobic core. When Gag binds on cytoplasmic membrane, the myristate is released and anchors the MA on the membrane. This is called myristoyl switch. In HIV-1 MA the myristoyl switch is regulated by phosphatidylinositol-4,5-bisphosphate (PIP), which is a phospholipide and is a part of the cytoplasmic membrane. PIP binds on HIV-1 MA and triggers the release of myristoyl, one of its fatty acid chains interacts with MA, strengthening the binding on the membrane. Because PIP is also necessary for HIV-2 and MMLV MA`s binding, we believe that PIP plays an important role in the interaction of M-PMV MA with the membrane.

We have studied the interaction of PIP with MA using NMR spectroscopy and artificial liposomes. For NMR measurement we used soluble PIP whith C_8 fatty acid chains. Upon titration of MA with an increasing concentration of PIP we observed changes of MA chemical shifts. Therefore, STD-NMR (saturation transfer difference) experiment was measured at different MA/PIP ratios.demonstrating that the major binding epitope is located on the fatty acid chains.. These experiments allowed us to prove that the interaction is specific and to calculate an approximate structure of the PIP-MA complex. We also prepared artificial liposomes from phosphatidilcholine with 10% of PIP and mixed them with MA. Bound MA was separated from the ubound molecules by ultracentrifugation.We have proven, that M-PMV MA interacts with PIP specifically and that the binding mode is copletely different from HIV-1 and HIV-2.

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L5

STRUCTURE DIFFERENCES BETWEEN PROTEIN CRYSTALS AND SOLUTION STRUCTURES MONITORED BY RAMAN SPECTROSCOPY – CASE STUDY OF PSBP PROTEIN

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Raman spectroscopy gives a unique opportunity to study protein samples in different phases. It is possible to measure intact protein crystals directly in hanging drops in the crystallization boxes they actually grow and to study the protein structure in crystals as well as chemical reactions in single crystals [1]. Moreover, we apply a new technique of Raman spectroscopy – a drop coating deposition Raman (DCDR) method [2], based on a coffee ring effect, that enables measurements of solutions down to 1 μM concentrations. However, our recent work adverted to subtle differences which correspond to the glass-like phase of the deposited samples [3]. Thus, DCDR protein samples represent a "phase transition" between saturated protein solutions and crystals. This enables to distinguish spectral differences given by the density of molecules in crystals from those caused by protein crystal artifacts.

Here we illustrate the new approach by Raman spectroscopy application on PsbP protein of photosystem II from *Spinacia oleracea* [4]. The protein is a part of the so-called oxygen-evolving complex, which is involved in photosynthesis. Recombinant PsbP protein was prepared as thrombin-digested recombinant His-tagged PsbP protein overexpressed in *Escherichia coli.* The crystal structure of PsbP protein from *Spinacia oleracea* was solved to the resolution of 2.06 Å [5]. Subsequently, Raman spectra of PsbP have been collected from solvent, from the DCDR deposit and from the crystal sample. Mutual comparison of these Raman spectra enabled to point on shortcomings in the crystal structure with respect to the structure in a solution. Moreover, these informations gave the possibility to model unresolved parts of the crystal [4].

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L6

STRUCTURE OF GUANINE OCTAMERS D(G)$_8$ DETERMINED BY COMBINATION OF VCD SPECTROSCOPY AND THEORETICAL COMPUTATIONS

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Despite the vast amount of spectroscopic data available up to date, the solution structure of polynucleotides and oligonucleotides rich in guanine bases remains ambiguous. Different geometries have been proposed, ranging from single- to multiple-stranded arrangements depending on the experimental conditions [1-4]. Being relatively simple and widely available, IR spectroscopy is a convenient method to study the structural organization of nucleic acids in solutions. Later, a more sensitive chiroptical derivative of IR spectroscopy, vibrational circular dichroism (VCD),

was successfully employed for such studies [1, 5]. However, the results obtained by these methods for polyG and similar systems significantly varied even at comparable experimental conditions. Fairly different IR and VCD spectra have been ascribed to four-stranded structures [1-4, 6, 7]. In some cases no explanation has been given for such discrepancies, while in the others the observed differences have been attributed to metastable polyG quadruplex structures [3, 4], or to a quadruplex-duplex transition [4].

To shed some light on the ambiguity exhibiting by the G-rich nucleic acid systems, we performed a combined experimental and computational study of the $d(G)_8$ octamer. Experimental IR and VCD spectra were measured for the octamer at standard conditions, used also in other studies devoted to G-quadruplexes. Theoretical IR and VCD spectra were calculated for single-, double- and quadruple-stranded $d(G)_8$ systems employing a multi-scale approach. The computational methodology included initial molecular dynamics (MD) simulations for each proposed structure, followed by ab initio calculations of force fields and atomic tensors for smaller fragments obtained from the octamers, and a subsequent transfer of those properties to the original octamers according to the Cartesian coordinate tensor (CCT) techniques [8]. On the basis of a comparison of the computed spectra with experiment the most probable structures could be determined.

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L7

CHARGE TRANSPORT IN DNA OLIGONUCLEOTIDES WITH VARIOUS BASE-PAIRING PATTERNS

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Charge migration in DNA has attracted a considerable amount of interest for its relevance to the mechanism of damage in nucleic acid and for its possible applications in the area of nanotechnology. We have combined various experiments and theoretical models to elucidate further the DNA charge transfer process in terms of DNA base pairing patterns, base stacking interaction and the role of the sugar-phosphate backbone. Through an exploration of the relation between the physical and chemical statuses of a particular DNA modification and its charge transport properties, we intended to affect the extremely complex charge transport process and its biological and technical significance. Our experimental data as well as the results from the theoretical models and calculations indicate that DNA charge transport strongly depends on the presence of structural perturbations, in particular irregularities in base-pairing and base-stacking patterns. Even seemingly unimportant structural perturbations caused by the presence of mismatched base-pairs affect conductivity to a greater extent than expected from conformational changes and decreased thermal stability alone.

Lectures - Thursday, March 18, Session II

L8

THE INTERFACE BETWEEN OXIDIZED PHOSPHOLIPID BILAYERS AND AQUEOUS SOLUTIONS

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Oxidation processes in membranes accompany various pathological conditions in organisms, nevertheless the influence of oxidation on the membrane structure is still poorly understood. Here we monitored the time evolution of a massively oxidized phospholipid membranes using molecular dynamics simulations. Oxidation leads to specific acyl chain reorientations. Massive oxidation also destabilized the membrane and, depending on its extent and type, in some cases even destroyed its structure. Creation of stable pores and consequent water penetration was observed as a direct consequence of oxidation. Here, we describe here the molecular mechanisms of this pore formation.

L9

AQUEOUS SALT SOLUTION OF ORGANIC SOLVENTS AS A MEDIA FOR ENZYMATIC REACTIONS

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The effect of some nonionizing, water-soluble organic solvents on the enzyme activity was studied both by experiments and molecular dynamics (MD) simulations. Also it has been shown that many mono or divalent cations and anions can enhance the activity of enzymes in aqueous solutions of organic solvents [1,2]. Organic solvents can have different effect of the catalytic reaction such as lowering the energy of activated complex or stabilization of enzyme in solution. Also using solution of organic solvents can improve the solubility of some reactant. For example in the case of haloalkane dehalogenase the mixture of organic solvent with water can increase the solubility of haloalkanes which have low solubility in water. In this work solvation and dynamics of some organic solvents in water have been studied by means of molecular dynamics (MD) simulations. Moreover the effect of mixture of some organic solvents such as DMSO, isopropanol and formamide on structure and dynamics of enzymes have been studied. Also it is found from MD data that in some cases solution of organic solvents prevent aggregation of some organic compound such as carotenoides in water.

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L10

EFFECT OF ORGANIC SOLVENTS ON STRUCTURE AND FUNCTION OF HALOALKANE DEHALOGENASES

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The enzymatic catalysis in pure organic solvents or their mixtures with water has developed into active area of research. The utilization of enzymes as highly selective biocatalysts in organic media is being widely applied in the synthesis of many enantiopure compounds [1]. Biochemical properties of enzymes, such as activity, specificity and stability, have been shown to be dependent on the choice of reaction medium [2]. However, not much is known about molecular mechanisms governing the effect of organic solvents on enzymes and their biochemical properties.

The aim of this project is to elucidate the behaviour of three haloalkane dehalogenases (LinB from *Sphingobium japonicum* UT26, DhaA from *Rhodococcus rhodochrous* NCIMB13064 and DbjA from *Bradyrhizobium japonicum* USDA110) in nonconventional media to optimize the conditions for their utilization in industrial applications and to develop enzymes that are catalytically active in the presence of organic solvents. Using kinetic experiments in the presence of various concentrations of thirteen organic solvents we demonstrate, that the tolerance of three studied enzymes to various organic solvents is different even though they belong to the same protein family. DhaA and DbjA are active in aqueous solution as well as in the presence of most of the organic solvents. Moreover, the selected organic solvents have even stimulatory effect on DbjA activity. On the contrary, activity of LinB was significantly decreased in most of the tested solvents. The connection between loss of activity and conformation changes, studied using circular dichroism and fluorescence spectroscopy, was found with DhaA and DbjA, while LinB structure remain mostly intact. To understand the mechanism of interaction of organic solvents with enzymes at the atomic level, the behaviour of LinB, DhaA and DbjA in water and three representative solvents was investigated using molecular dynamics simulations. It was found, that molecules of inhibiting solvents enter the active site in larger number and form a solvent network, while activating solvents are present in active site as a single isolated molecule. The access of solvent molecules to the active site seems to be determined by physico-chemical properties of walls of the tunnel and/or the active site. Kinetic inhibition analysis of enzymes in organic solvents is currently on-going in our laboratory to experimentally verify observations from modelling.

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L11

STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF NOVEL HALOALKANE DEHALOGENASE DBEA FROM *Bradyrhizobium elkani* USDA94 REVEALED TWO HALIDE BINDING SITES IN HALOALKANE DEHALOGENASES

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A novel haloalkane dehalogenase DbeA belonging to the subfamily HLD-II [1] was isolated from soil bacteria *Bradyrhizobium elkani* USDA94. This new enzyme is closely related to DbjA from *Bradyrhizobium japonicum* USDA110 [2]. Proper folding of DbeA was assessed by measurement of CD spectra in far-UV and near-UV spectral regions. Thermal stability of DbeA was evaluated by determination of the melting temperature (Tm = 58.5 ± 0.2 °C), which is in the similar range as structure stability observed for other family members. Molecular weight determined by gel filtration and native polyacrylamide gel electrophoresis confirmed dimeric state of DbeA under native conditions. Activity data of HLDs were measured with a set of 30 various substrates. The principal component analysis of the specific activities showed that DbeA is less active than DbjA and posses a unique substrate specificity. This enzyme has the highest activity towards brominated and iodinated compounds from all tested HLDs. DbeA showed high enantioselective conversion of 2-bromopentane, 2-bromohexane and brominated ester of propionic and butyric acid into chiral alcohols. The temperature and pH profiles of DbeA were detected by activity measurement with 1-iodohexane as a substrate. The highest activity of the enzyme was detected at the temperature range 45-55 °C, which is in a good agreement with the temperature profiles of other HLDs. Surprisingly, DbeA showed more than one pH optimum with the maximal activity detected at pH conditions 6.0 and 8.5-9.5. Two pH optima were described only for DmbA, while other HLDs exhibited single pH optimum.

Crystallographic analysis of DbeA revealed the presence of two halide binding sites for chloride anion. The first chloride anion in DbeA structure was found in product-binding site where interacts with conserved halide binding residues Asn38 and Trp104. This binding site is common for all HLDs-II. The second chloride anion in DbeA structure is placed about 10 Å far from the product-binding site, buried deep in the protein core, where is coordinated by side chains of Gly37, Thr40, Ile44, Gln102 and Gln274. This chloride-binding site is unique to DbeA and its closely related enzyme DbjA. The full occupancy of this second chloride binding site and its location in close proximity of the active site suggests that this halide-binding site might have some biological relevance, perhaps on DbeA activity. To elucidate the role of the second halide binding site on DbeA structure and function, the two point mutant variant lacking the second binding site, DbeA I44L and Q102H, was constructed and characterized. The comparison of the wild type and mutant enzymes will be presented and discussed.

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L12

WAS BINDING OF FREE AMINO ACIDS AN EARLY INNOVATION IN THE EVOLUTION OF ALLOSTERY?

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Interpretation of thermodynamic ligand-binding data through the lens of molecular dynamics (1) has led to a structural and energetic description of the molecular mechanism of allostery for the hexameric *E. coli* arginine repressor, the master feedback regulator of transcription in L-arginine metabolism, which displays strong negative cooperativity of L-arginine binding. A controversial prediction of the famous allostery model of Monod, Wyman, and Changeux is that constraints imposed on protein subunits by multimerization are relaxed by ligand binding, but with conservation of symmetry in partially-liganded states. Molecular dynamics simulations reveal that conserved Arg and Asp sidechains in each L-arginine binding pocket promote rotational oscillation of apoArgR trimers by engagement and release of salt bridges. Binding of exogenous L-arginine displaces resident Arg residues and arrests oscillation, shifting the equilibrium quaternary ensemble and promoting subunit motions that generate an entropic driving force while maintaining symmetry in partially-liganded states.The results indicate that partially-liganded states can be structurally symmetric despite their conceptual asymmetry. The symmetric relaxed state is visualized as a multimer with all subunits anchored near the center, and with motions transferred to the periphery like a bouquet of balloons in strong wind. Thus, even during sequential filling of binding sites, symmetry can be maintained by exploiting the dynamics of the assembly and the distributed nature of its cohesive energy. The mechanism suggests the possibility that binding of free amino acids was an early innovation in the evolution of allostery (2).

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