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Abstracts

A1

VIBRATIONAL CIRCULAR DICHROISM STUDY OF ISOTROPIC AND LIQUID CRYSTALLINE PHASES FORMED BY GUANINE QUADRUPLEXES

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One of the preferred conformations of guanine-rich nucleic acids in aqueous solutions is a four-stranded G-motif. Both oligonucleotides and monomeric guanosine monophosphates can form G-quadruplex structure at certain experimental conditions. At lower concentrations they represent an isotropic solution of four-stranded systems. However, above certain concentration these structures can self-associate and form liquid crystalline phases. Depending on the concentration two types of assemblies can be distinguished, cholesteric and hexagonal. The optical behaviour of these supramolecular systems can be most conveniently monitored in the infrared region by vibrational circular dichroism (VCD) spectroscopy. An abnormally high VCD intensity was observed for both liquid crystalline phases compared to the isotropic solution. The direction of the helical twist was obtained for guadruplexes as well as for their supramolecular aggregates.

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A2

HIGH THROUGHPUT SCREENING OF POTENTIAL GCPII INHIBITORS BY FLUORESCENCE POLARIZATION

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Glutamate Carboxypeptidase II (GCPII) inhibition is an important aim for current research on diagnostic and therapeutic interventions inprostate cancer and neurologic disorders. Here we describe the development and optimization of a High Throughput Screening (HTS) assay, based on fluorescence polarization (FP), to identify new scaffolds inhibiting GCPII. The synthesis of a fluorescence probe was accomplished by covalently attaching a Bodipy TMR fluorophore to a urea-based GCPII inhibitor. The conditions for HTS and robustness of a FP based assay were optimized considering factors as pH, temperature, time and additives. The approach showed suitability to detect both competitive and non-competitive GCPII inhibitors and the results are comparable to those provided by benchmark assays. Thereafter, the assay was used to screen a library of 20,000 pharmacologically active compounds. The determination of a Z' factor 0.82 validates the performance of the assay in a high-throughput format. It additionally represents a non-hazardous, inexpensive and robust methodology to identify novel GCPII inhibitors.

A3

CHARACTERIZATION OF N-TERMINAL PART OF HUMAN RYANODINE RECEPTOR 2

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The ryanodine receptor (RyR) is the calcium release channel of a vital importance, responsible for muscular contraction in mammalian cells. It is localized on the membrane of the sarcoplasmatic reticulum (SR), where it transfers Ca^{2+} from SR into cytoplasm. This channel is composed of a larger cytoplasmic and a smaller transmembrane part and is built up from four polypeptide chains to give a homotetramer with a molecular weight of ~ 500 MDa. Due to its size and properties, the structure of the whole receptor has been determined only by cryoelectron microscopy [1,2]. An X-ray crystal structure has been determined for only the first 217 aa. [3] and for the first 532 residues of RyR1 [4].

In this work we have focused predominantly on the domain analysis of the N-terminal region (residues 1–759) of the human cardiac ryanodine receptor (RyR2) which includes RyR2 mutation cluster associated with catecholaminergic polymorphic ventricular tachycardia (CPVT1) and arrhythmogenic right ventricular dysplasia (ARVD2). In our strategy we used a bioinformatics approach followed by protein expression, solubility analysis and limited proteolytic digestion [5,6]. Based on the bioinformatics analysis, we designed a series of specific RyR2 N-terminal fragments for cloning and overexpression in *Escherichia coli*. High yields of soluble proteins were achieved for several N-terminal RyR2 fragments. The purity, monomeric state, folding and stability of these proteins were verified by SDS PAGE, gel filtration, dynamic light scattering measurements, Circular dichroism and thermofluor shift assay. Moreover, based on the similarity between RyR1 and RyR2 isoforms, we performed a homology modeling of the N-terminal fragment of RyR2 (aa. 1 – 553). All these approaches help us to understand better structure-function relationship of this protein.

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A4

ENANTIOSELECTIVE CONVERSION OF 2,3-DICHLOROPROPANOL BY HALOALCOHOL DEHALOGENASE HHEC: DESIGN OF NON-SELECTIVE CATALYST

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TCP is widely used persistent contaminant with a carcinogenic and toxic effect on living organisms [1]. The conversion of 2,3-dichloropropanol (DCP) to corresponding epoxide is the second step in the dechlorination of 1,2,3-trichloropropan (TCP) to glycerol [2]. The haloalcohol dehalogenase HheC [3] catalyses this second step in the TCP biodegradation pathway by cleaving-off the two remaining chlorine atoms of the DCP. However, the enantioselectivity of this reaction represents significant problem in the biodegradation pathway since after convertion of (*R*)-DCP, toxic (*S*)-DCP accumulates in the solution.

Only limited knowledge about molecular principles of HheC enantioselectivity is available to date. Molecular docking and quantum mechanics calculations were therefore employed to provide detailed information about molecular basis of HheC enantioselectivity. Molecular docking revealed small preference in binding of *(R)*-DCP over (*S*)-DCP, which corresponds well with experimental analysis. The two-step dehalogenation reaction was proposed for conversion of DCP based on quantum mechanical calculations, but no significant difference in the conversion of (*R*)- and (*S*)-DCP was observed at the level of employed theory. Results from molecular docking and quantum mechanics were complemented by FoldX calculations [4] to identify several hot spots for saturation mutagenesis. Experimental construction of mutants and their screening for enantioselectivity is currently on-going in our laboratory.

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A5

THE INFLUENCE OF THE C-TERMINAL HELICAL DOMAIN ON ATPASE ACTIVITY IN THE RESTRICTION-MODIFICATION COMPLEX ECOR124I

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Restriction-modification enzymes (R-M) protect bacteria from infections by viruses, and it is commonly accepted as being their major role in nature, thus they function as the main players constituting microbial immune systems [1]. The phenomenon of restriction, identified first for type I R-M systems, laid the foundations for modern molecular biology, and, eventually, led to the discovery of widely used in DNA cloning techniques and highly commercialized type II restriction enzymes. The classical R-M systems of *Escherichia coli* K-12 and *E.coli* B were first to be discovered by Bertani and Weigle back to 1953 [2]. The classical type I R-M enzymes of *E. coli* K-12 (EcoKI) and B (EcoBI) were not only the first to be detected but also the first to be purified [3, 4].

Type I R-M enzymes are large, multifunctional macromolecular complexes composed of three different subunits: HsdS, HsdM and HsdR [5]. The activities of the complex of all three subunits include ATP-dependent DNA translocation, DNA cleavage and methylation [6-9].

HsdR is organized into four approximately globular structural domains in nearly square-planar arrangement: the N-terminal endonuclease domain, the recA-like helicase domains 1 and 2 and the C-terminal helical domain. The near-planar arrangement of globular domains creates prominent grooves between each domain pair. The two helicase-like domains form a canonical helicase cleft in which double-stranded B-form DNA can be accommodated without steric clash. A positively charged surface groove proceeds from the helicase cleft and continues between the helical and endonuclease domains where it passes over the cleavage site recessed slightly from the surface. The helical domain resembles HsdM and has strong interactions with helicase 2 domain [10].

In the present work we test mutations, which could be essential for helical-helicase 2 domains interactions, by using a combination of site-directed mutagensis and *in vivo* and *in vitro* restriction activity assays. Absence of these interactions influences either subunit assembly, rotation of helicase 2 domain relative to helicase 1 domain, loading of dsDNA in the helicase cleft or DNA translocation and following restriction.

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A6

INVESTIGATION OF LIGAND PASSAGE IN PROTEINS

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Many enzymes have buried active sites, in other words, the active site is not located on the protein surface, but deep within the protein structure, and is connected to the bulk solvent with one or more tunnels. The properties of these tunnels affect ligand binding and unbinding and, therefore, also the catalytic properties (activity, substrate specificity) of the enzyme. The model system we use in our study is the haloalkane dehalogenase (HLD) enzyme LinB. The HLDs are bacterial enzymes with great potential in various biotechnological applications including biocatalytic production of optically pure compounds, and biosensing and biodegradation of toxic environmental pollutants. Optimization of these enzymes' catalytic activity by mutagenesis would help to improve the economic feasibility of such applications. We have investigated ligand passage in the LinB enzyme, and the effect of introducing a bulky tryptophan at a tunnel-lining position. Transient kinetics experiments showed that the mutation significantly slows the rate of product release. Moreover, the rate of bromide ion release corresponds to the overall steady-state turnover rate, suggesting that product release became the rate limiting step of the mutant catalysis. We performed classical molecular dynamics (MD) simulations of both the wild type LinB and the mutant. Analysis of trajectories from MD simulations with the Caver 3.0 program revealed differences in the tunnels available for ligand egress. Corresponding differences were observed in simulations of product egress using the random acceleration molecular dynamics (RAMD) technique. The RAMD results are in qualitative agreement also with experimental observations of the binding and unbinding rates of the ligands studied. We then calculated free energy profiles for the key product, bromide, along the preferred tunnel in both wild type LinB enzyme and in the mutant. For this purpose, we used the Adaptive Biasing Force (ABF) method. The differences in the free energy barriers for egress of a bromide ion calculated using ABF are in good agreement with the differences in rates obtained from the transient kinetic experiments. Detailed analysis of the ABF trajectories enabled to identify specific molecular interactions responsible for the higher free energy barrier for bromide unbinding in the mutant. The study demonstrates that the mechanisms of individual steps of the catalytic cycle can be engineered by modification of the enzyme's tunnels.

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A7

CANONICAL TRANSIENT RECEPTOR POTENTIAL CHANNEL TRPC6 AND S100A1 PROTEIN AS BINDING PARTNERS

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S100A1 is a calcium-binding protein which undergoes large conformational change upon binding calcium interacts with numerous target proteins involved in signaling pathways e.g. calcium signaling, neurotransmitter release or cooperation with cytoskeletal and filament associated proteins. Superfamily of TRP channels can function as calcium influx channels in eukaryotic cells, sensory receptor cells and play role in response to external signal. TRPC6 is Ca^{2+} permeable non-selective cation channel and its activity is modulated by multiple factors (calmodulin, PIP_2 etc).

We indentified the binding site for S100A1 protein on C-terminus of the transient receptor potential from canonical subfamily TRPC6 by using methods of fluorescence anisotropy and surface plasmon resonance. Part of C – terminus of TRPC6 (amino acids 801-878) was subcloned in PET42b expression vector with histidine tag. Point mutations of several amino acids residues for alanine were performed. Recombinant protein TRPC6 and all mutants were purified using chelating Sepharose fast flow and by HPLC chromatography (Superdex 75). The cluster of basic charged amino residues R852A 859A R860A R864A was involved in the binding of S100A1 protein on basis the fluorescence anisotropy measurements. It can be concluded that putative bunding site on TRPC6 C- tail binds S100A1 in a similar way like another calcium binding protein calmodulin.

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A8

THE STUDY OF HUMAN RYANODINE RECEPTOR 2 N-TERMINAL REGION RESPONSIBLE FOR HEART DYSFUNCTION

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The ryanodine receptor 2 (RyR2) is the calcium release channel responsible for contraction in mammalian myocardium cells. It is a large homotetramer, composed of four subunits with a molecular weight of ~560 kDa [1] and it is localized in the membrane of sarcoplasmatic reticulum [2]. The main RyR2 opening/closing regulation mechanism is considered to be the interaction between the N – terminal (aa ~ 1-600) and central region (aa ~ 2100-2500) [3] in the cytoplasmatic part. A hypothesis was proposed that mutations in these interacting regions cause regulation failure and thus lead to nonspecific Ca^{2+} release which results in several heart diseases [4].

Prediction performed by PFAM database revealed the presence of 14 domain in the RyR2 molecule [5]. In our work we have predominantly focused on the RyR2_1-606 and RyR2_1-655 fragments which involve three putative PFAM domains: Ins145_P3_rec, MIR, RIH [5,6] as well as several mutantions of RyR2_1-606 which may cause heart failure. The purity and solubility of RyR2_1-606 and RyR2_1-655 were verified by gel filtration and SDS PAGE. Monodispersity and stability in time was confirmed by dynamic light scattering (DLS). Thermofluor shift assay was used to find the conditions of highest thermal stability. Circular dichroism spectra [5,6] as well as preliminary thermofluor shift assay results indicate that the protein is folded and starts melting at around 40°C. Obtained purity, solubility and stability of RyR2_1-606 and RyR2_1-655 allow us to apply structure determining approaches: small angle scattering (SAXS) and X-ray crystallography, which could contribute to the RyR2 structure-function relation study.

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A9

QSPR MODELS PREDICTING PK_A FROM ATOMIC CHARGES

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The acid dissociation constant pK_a is one of the fundamental properties of organic molecules. The pK_a values are of interest in chemical, biological, environmental and pharmaceutical research. One critical feature is that pK_a values help discover whether molecules can be used as drugs. Moreover, pK_a values are essential for ADME profiling, give insight into interactions of drugs with a receptor, etc..

Several methods for pK_a calculation have been developed [1], but prediction of pK_a values remains a challenge. A very promising approach for pK_a prediction is the usage of QSPR (Quantitative Structure Property Relationship) models which employ partial atomic charges [2,3,4]. The accuracy of pK_a prediction by these QSPR models is influenced by many factors. Very important factors are proper selection of descriptors, usage of relevant charge schemes, influence of a molecular structure, etc..

In our work, we analyzed the key factors which influence the quality of QSPR models for pK_a prediction. We focused on three types of molecules – phenols, benzoic acids and anilines. For each type of molecules, we studied the influence of the charge calculation scheme on the quality of QSPR models. Specifically, we tested combinations of three QM theory levels (HF, MP2 and B3LYP), three basis sets (STO-3G, 6-31G* and 6-31+G*) and three population analyses (Mulliken, MK and NPA). This evaluation was, in most of cases, done for three sets of descriptors – charges from non-dissociated molecules, charges from dissociated molecules, and a combination of both these types of charges. Afterwards, we compared the accuracy of all these QSPR models and discussed the influence of all factors.

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A10

VISUALIZATION OF ATOMIC CHARGES IN MOLECULES: COMPARISON OF AVAILABLE APPROACHES AND SOFTWARE TOOLS

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Partial atomic charges are real numbers describing the proportion of electronic density which belongs to each atom in a molecule. They are created due to the asymmetric distribution of electrons in chemical bonds. The charges provide very useful information for chemists and biochemist and therefore have a lot of applications [1, 2, 3]. For example, they are very effective descriptors in QSAR/QSPR models for prediction of dissociation constants, partition coefficients and other important physico-chemical properties. Charges are also employed in molecular mechanics and dynamics simulations, because they are necessary for calculating the electrostatic part of the potential energy. Docking and virtual screening also use charges for calculating electrostatic interactions. Atomic charges can be also used to estimate the chemical reactivity of molecules.

Especially nowadays partial atomic charges became very popular in chemoinformatics, because advanced computational methods and high performance computers allow us to obtain them quickly even for large sets of molecules [3, 4, 5]. This increased usage of charges generated a demand for their visualization by representing the charge distribution over a molecule in a manner that is both accessible and intuitive for humans. There are several models for visualization of charges, each having its advantages

and disadvantages (text labeling, coloring of individual atoms or surfaces). The increasing popularity of charges caused that several software tools for visualization of molecules (i.e. VMD, Jmol, DSV, Mol2mol, Maestro) to extended their functionality and add visualization of charges.

In the presented study, an overview of available charge visualization approaches is provided. Afterwards, selected softwares packages are evaluated regarding their ability to visualize atomic charges. The evaluation was performed for different types of molecules (small organic molecules, peptides, biomolecules) and using different visualization models. The study includes the comparison of rendering quality and time, various visualization options, data format support and software accessibility.

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A11

ANALYSIS OF BIOMOLECULAR INTERACTIONS BY SURFACE PLASMON RESONANCE

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Surface Plasmon Resonance (SPR) is a physical process that can occur when plane-polarized light hits a metal film under total internal reflection conditions. This phenomenon is employed as an excellent technique to measure biomolecular interactions in real-time in a label free environment. In this method, one of the interacting partners (ligand) is immobilized to the sensor surface, while the other (analyte) is free in solution and passed over the surface. The kinetic parameters of the interactions are further calculated from association and dissociation curves. This lecture will introduce a SPR optical biosensor "ProteOn XPR36 protein interaction array system" recently installed at the Institute of Microbiology in Prague. This system generates a 6 x 6 interaction array for the simultaneous analysis of up to six ligands with up to six analytes which enables analysis of up to 36 different protein interactions in a single run on a single chip. Few examples, such as protein-protein interaction of adenylate cyclase toxin (CyaA) with integrin receptor CD11b/CD18, lipid-protein interaction of phosphatidylinositol 4,5-bisphosphate (PIP2) with membrane channel TrpV1, or DNA-protein interaction of transcription factor FoxO4 with target DNA, will be presented to show the throughput, flexibility and versatility of this instrument.

A12

COMPLEMENTARY STRUCTURAL INVESTIGATION OF INTRINSICALLY DISORDERED PROTEIN TAU, INVOLVED IN NEURODEGENERATIVE DISEASES

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Microtubule-associated intrinsically disordered protein tau has under physiological conditions very flexible molecule, similar to the random coil [1]. When interacting with microtubules, the proline-rich and repeat regions of tau molecule play the most prominent role [2]. In the course of neurodegenerative tauopathy (e.g. Alzheimer's disease), tau undergoes numerous posttranslational modifications, dissociates from microtubules and forms highly compact, insoluble paired helical filaments (PHF) [3]. Interestingly, microtubule-interacting sequences on tau constitute the core of the PHF [4].

In our work, the thermodynamics and structure of microtubule binding motifs of tau have been studied with the use of monoclonal antibodies as surrogate tau protein binding partners. Surface plasmon resonance was used to monitor the binding of monoclonal antibody Fab fragment to full length and truncated tau proteins. Thermodynamic analysis showed that specific truncation of tau protein molecule, which was observed under disease conditions [5, 6],

results in substantial alteration of tau binding to antibody, pointing to higher accessibility of microtubule binding sequences in truncated tau proteins. To make an insight into structure of tau complexes, Fab fragments have been crystallized alone and with tau peptides. We report preliminary X-ray diffraction analysis of three crystal types diffracting up to 1.72 Â.

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A13

CAVER 3.0: A TOOL FOR ANALYSIS OF TRANSPORT PATHWAYS IN DYNAMIC PROTEIN STRUCTURES

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Tunnels and channels mediate the transport of ions and molecules in a large variety of proteins [1-4]. Characteristics of individual transport pathways, including their geometry, physico-chemical properties and dynamics, are essential for understanding of structure-function relationships of these proteins, and for the design of improved biocatalysts or new inhibitors [5]. Here, we present CAVER 3.0, a new tool for the geometry-based analysis of pathways in protein structures. CAVER 3.0 was designed to facilitate the analysis of static crystal structures as well as dynamical systems, and for this purpose it implements several new algorithms for calculation and clustering of pathways. In the input, users provide the protein structure or a set of aligned structures and specify calculation starting point. A number of additional settings are available, enabling users to adjust the calculation based on their needs. In the output, CAVER 3.0 provides all necessary data for the analysis of the time evolution of identified pathways, including the scripts for opening of the results in structure visualization software, information about pathway characteristics, amino acid composition and pathway profiles. Altogether, CAVER 3.0 enables an effective analysis of pathways in dynamic protein structures, which opens up new possibilities for the study of important biochemical phenomena in the area of molecular transport, molecular recognition and enzymatic catalysis. The software is freely available as a multiplatform command-line application at http://www.caver.cz.

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A14

CHARACTERIZATION OF NOVEL HALOALKANE DEHALOGENASE ISOLATED FROM PSYCHROPHILIC BACTERIUM *MARINOBACTER* SP. ELB17

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Haloalkane dehalogenases (EC 3.8.1.5) catalyse hydrolytic conversion of halogenated aliphatic hydrocarbons to their corresponding alcohols and halide anions. Since the first member of the family was isolated [1], haloalkane dehalogenases became widely studied due to their potential application in biocatalysis, biosensing of environmental pollutants and biodegradation of toxic compounds. Searching for new enzymes has attracted the interest of many enzymologists and companies, which look for biocatalysts suitable for biotechnological and pharmaceutical applications. Extremophilic organisms offer opportunities for finding novel enzymes exhibiting unique properties. The principal advantage of exploitation of such enzymes is their high catalytic efficiency under extreme conditions.

This study is focused on cloning, expression, purification and biochemical characterization of novel haloalkane dehalogenase DmxA isolated from psychrophilic bacterium *Marinobacter* sp. ELB17. Synthetic *dmxA* gene in pMA vector (Mr. Gene, Germany) was subcloned into pET21b expression vector. DmxA was expressed in *Escherichia coli* BL21(DE3) cells and purified to homogeneity by metalloaffinity chromatography. Correct folding and thermostability of DmxA was assessed by circular dichroism spectroscopy. Compared to other haloalkane dehalogenases, DmxA exhibited, paradoxically, the highest melting temperature (T_m = 65.9 ± 0.1 °C). Substrate specificity of the enzyme was measured with thirty different halogenated substrates. DmxA was the most active towards 1,3-dibromopropane, 1-bromo-3-chloropropane and 4-bromobutyronitrile. Temperature and pH profiles of DmxA were determined with 1,3-dibromopropane by activity measurement. Maximal activity was detected at 55 °C and at pH 9.0. Steady-state kinetic parameters of DmxA were measured with 1,3-dibromopropane and 1-chlorobutane. The complex kinetic mechanism of the enzyme was determined, involving cooperativity and substrate inhibition. Enantioselectivity of DmxA was tested towards selected -substituted bromoalkanes and brominated esters. High enantioselectivity of DmxA was observed in the reaction with ethyl-2-bromobutyrate (E > 200), methyl-2-bromobutyrate (E > 200) and 2-bromopentane (E = 100). The unique properties, as high thermostability and enantioselectivity, make DmxA suitable catalysts for practical application in biosynthesis of optically pure compounds.

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A15

SPOIIE LINKS ASYMMETRIC CELL DIVISION TO COMPARTMENTALIZATION OF GENE EXPRESSION DURING SPORULATION OF *BACILLUS SUBTILIS*

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Sporulation in *B. subtilis* serves as an example of primary cellular differentiation. Daughter cells arisen from sporulation division follow the different fate, although they share identical genetic information. The process of sporulation is characteristic by an asymmetric cell division, which results in formation of two disimilarly sized compartments, the smaller prespore, and the bigger mother cell. The proper positioning of an asymmetric septum is partially triggered by sporulation specific SpoIIE, an exclusive component of the sporulation septum. During formation of sporulation septum, SpoIIE closely cooperates with tubuline-like protein FtsZ. FtsZ forms higher organized structures, so called Z-rings, which constitute a scaffold for assembly of division machinery. During vegetative division, there is only one Z ring formed and it is positioned at the midcell. However, during sporulation, FtsZ is over-ex-

pressed and forms a helical structures emanating from midcell Z ring towards cell poles. SpoIIE, which also forms ring-like structures, most likely stabilizes FtsZ helices at polar positions [1]. Consequently, two Z-rings are formed at the sites near cell poles, but only one is chosen to become the sporulation septum.

SpoIIE is a multi-domain 827-residue protein. It is thought to consist of three domains. Its N-terminal domain, which consists of 10 transmembrane helices, anchors the protein in sporulation septum [2, 3]. The central domain of SpoIIE, which is conserved only among SpoIIE orthologues, is thought to play a role in self-oligomerization of the protein and it is also responsible for interaction with FtsZ [4]. SpoIIE C-terminal domain is closely related to PP2C domains of eukaryotic Ser/Thr phosphatases, which regulate the stress response [5].

The key regulator SpoIIE has probably three roles in the process of sporulation. The first role of SpoIIE is to stabilize FtsZ spiral intermediates at polar positions. The second role of SpoIIE lies in contribution to prespore-specific ^{F} activation. ^{F} is activated as the first of sporulation compartment-specific sigma factors and its activation is linked to the completion of sporulation septum [6]. Consequent compartment specific gene expression is arranged by the activity of cell-type specific factors, which sequentially activate each other in a cascade-like manner. The third role of SpoIIE is unclear, but according to the dynamic localization studies using SpoIIE-GFP fusions following cytokinesis, SpoIIE might play a role in the process of engulfment [7].

Although ^{F} becomes active only in the prespore, it is present in the predivisional cell, and after asymmetric division also in both compartments. The activity of ^{F} is regulated through interactions between an anti-sigma factor SpoIIAB, an anti-anti-sigma factor SpoIIAA, and SpoIIE phosphatase, resident in sporulation septum. In the predivisional cell and in the mother cell, SpoIIAA is bound by SpoIIAB kinase, which results in phosphorylation of SpoIIAA and SpoIIAB free to form a SpoIIAB2: ^{F} complex. In this complex, ^{F} is inactive. In the prespore, SpoIIE dephosphorylates SpoIIAA-P, making it able to attack SpoIIAB2: ^{F} complex. By binding of dephosphorylated SpoIIAA to SpoIIAB, the release of active ^{F} is induced. ^{F} is then free to bind to the core of RNA-polymerase and direct transcription of prespore-specific genes [8,9]. The mechanism that delays ^{F} activation until sporulation septum is formed and confines ^{F} activity specifically into prespore is not yet fully understood. To address these issues, several models were suggested, taking into consideration the volume difference between the compartments, the preferential localization of SpoIIE to the forespore face of the septum, and/or transient genetic asymmetry [10-13].

The structures of the SpoIIA proteins have been determined, revealing the phosphorylated and dephosphorylated forms of SpoIIAA [14,15], as well as the interactions of SpoIIAB with ^{F} and SpoIIAA [9,16]. However, the structure of SpoIIE and the character of its interactions with SpoIIAA and FtsZ are still unknown, due to the problematic solubility of SpoIIE. Previously, using a random truncation library approach [17], a set of soluble SpoIIE fragments was identified. Out of these fragments, SpoIIE fragment (590-827) encompassing the PP2C phoshatase domain was over-expressed, characterized, and its crystal structure was determined [18]. Since SpoIIE has been a subject of intensive studies, a large number of *spoIIE* mutants have been characterized. Some of these mutations, like *spoIIE64, spoIIE71* and *spoIIE20*, are located within the phosphatase domain [19] All of them prevent activation of ^{F}, resulting in the inhibition of sporulation in cells harbouring these alleles. Cells of *spoIIE64* and *spoIIE 71* strains form normal asymmetric septa, but are defective in dephosphorylating SpoIIAA-P and hence activation of ^{F}. Interestingly, *spoIIE 20*, although it is mapped in the phosphatase domain, is impaired not only in activation of ^{F}, but also in formation of sporulation septa [19]. This mutation shows how the defect of phosphatase domain, which is responsible for ^{F} activation, can impair the central domain, responsible for morphological function of SpoIIE. This possibly happens by the means of interactions between the central and phosphatase domain of the protein. The structure of the phosphatase domain of SpoIIE provides a useful frame for interpreting genetic data available for this protein and for formulating ideas about its mechanism of action.

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A16

COMPLETE BASIS SET EXTRAPOLATION AND HYBRID SCHEMES FOR GEOMETRY GRADIENTS AND VIBRATIONAL ANALYSIS OF NONCOVALENT COMPLEXES

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This work focuses on the performance of popular WFT (MP2, MP2.5, MP3, SCS(MI)-MP2, CCSD(T)) and DFT (M06-2X, TPSS-D) methods in optimizations of geometries of noncovalent complexes. Apart from the straightforward comparison of the accuracy of the resulting geometries with respect to the most accurate, computationally affordable, reference method, we have also attempted to determine the most efficient utilization of the information contained in the gradient of a particular method and basis set. Essentially, we have transfered the ideas successfully used for noncovalent interaction energy calculations to geometry optimizations.[1]

We have found that CCSD(T) geometries are most faithfully reproduced by the MP2.5 and MP3 methods, followed by the comparably well performing SCS(MI)-MP2 and MP2 methods, finally by the worst performing DFT-D and M06 methods.

Basis set extrapolation of gradients was shown to improve the results and can be considered as a low-cost alternative to the use of CP-corrected gradients. A hybrid gradient scheme was shown to deliver geometries close to the regular gradient reference. Analogously to a similar hybrid scheme, which nowadays is routinely used for the calculation of interaction energies, such a hybrid gradient scheme can save a huge amount of computer time, when high accuracy is desired.

We will also present further extension of the highly accurate hybrid and CBS extrapolated gradients schemes for calculation of numerical vibrational frequencies of isolated molecules as well as their noncovalent complexes.

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A17

IDENTIFICATION OF POTENTIAL INHIBITORS OF HALOALKANE DEHALOGENASES BY VIRTUAL SCREENING

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Haloalkane dehalogenases (EC 3.8.1.5) are microbial enzymes which belong structurally to the hydrolase fold. They are able to degrade a broad range of halogenated aliphatic hydrocarbons. However, their biological function still remains unknown with the exception of some particular strains.

An effective inhibitor of these enzymes may help to decipher their function. In this project, we applied AutoDockVina docking software to screen the library of more than 140,000 compounds of the clean drug-like subset downloaded from the ZINC database . For the detailed analysis, 10,000 molecules with the lowest docked energies were selected, and their docked conformations were rescored by a neural network-based scoring function NNScore 2.0 . Additionally, their binding free energy was estimated using Molecular-Mechanics/Generalized-Born Surface Area (MM/GBSA). A consensus score for each docked ligand was then calculated by averaging the ranks

obtained from MM/GBSA and NNScore 2.0 score. To increase the diversity of potential inhibitors, software AuposSOM was employed to cluster the inhibitors accordingly to their common interactions with the enzyme. Finally, 100 top-ranked molecules were selected for experimental validation of their inhibitory activity.

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A18

THE C-TYPE LECTIN LIKE FOLD IN NATURAL KILLER CELL RECEPTORS

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Natural killer cells of the innate immune system recognize and degrade virally infected or tumour cells [1]. One significant group of NK surface receptors involved in this function are C-type lectin like (CTL) molecules involved either in activation or inhibition of the NK cell response. Receptor-ligand interactions are assumed to trigger a cascade of events in an NK cell, leading amongst others to the target cell lysis.

The mammalian receptors of the NKR-P1 family possess the extracellular part formed by a CTL domain, a neck region, a transmembrane region formed by a helix and an intracellular part responsible for signaling. The CTL fold of the extracellular domain is utilized also by the protein ligands of these receptors found on the surface of the encountered and recognized cell. In the light of some recent results predominantly protein-protein interactions are in the centre of such contacts. This implies that in the case of NK CTL receptors (or ligands) this simple fold is utilized for mediation of protein-protein contacts.

Interestingly, the CTL fold has been observed as a basic building block of many other proteins, such as the macrophage mannose receptor, selectins, collectins and others [2]. The structure of the CTL domain is characterized by two -helices, two small -sheets and a long surface loop. Two or three disulfide bridges increase the stability of the structure. Even if the main characteristics of the fold remain unchanged and mostly the long loop region is involved in performing the protein's function, several different mechanisms of exerting the functionality are employed. For some CTL proteins calcium binding in the long loop region is crucial for their affinity to ligands whereas in NK CTL receptors the same region is very likely incapable of calcium binding and at the same time involved in protein-protein interactions.

Our recent structural results suggest that the NK receptor ligands likely follow the fold, stability and dimerization pattern of the long known NK receptor human CD69 while the receptors themselves probably acquire more diverse structural arrangements [3, 4].

Interpretation of our X-ray structures of mouse NKR-P1A and Clrg, and other receptors in the context of other known CTL receptor structures, complemented by computational analysis and a critical view of results of other laboratories is expected to provide the so far most comprehensive view of structure-function features of these receptors and the specific utilization of the CTL fold in this case.

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