Posters

P1

MOLECULAR DYNAMICS STUDY: CATIONIC PHOSPHORAMIDATE -OLIGONUCLEOTIDES TARGETING SINGLE-STRANDED DNA AND RNA EFFICIENTLY STABILIZED BY BUTYL AMINO/GUANIDIUM TETHERS

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Much effort has been invested in recent years in the development of novel approaches aimed at the specific suppression of unwanted gene expression leading to viral and malignant diseases. The sequencing of the human genome and the elucidation of many molecular pathways that are important in disease have provided unprecedented opportunities for the development of new therapeutics. So-called „antisense“ oligonucleotides, inhibiting gene expression by creation of a helical complex with target mRNA (carrying ”sense” genetic information), represent a perspective approach in chemotherapy. A potential means to improve the efficacy of steric-blocking antisense oligonucleotides is to increase their affinity for a target RNA. The grafting of cationic amino groups to the backbone of the ON is one way to achieve this. Cationic -ON with phosphoroamidate internucleoside linkages incorporated into ON with a non-natural -anomeric configuration bound with high affinity to single-stranded DNA and RNA targets [1]. Duplex stabilization is proportional to the number of cationic modifications, with fully cationic ON having particularly high thermal stability. The average stabilization is greatly increased at low ionic strength. Cationic -ON are well suited as steric-blocking antisense agents as demonstrated in the case of hepatitis C virus [1]. Interestingly, no vectorisation was necessary for the cationic -ON in the cell culture.

The present work deals with cationic -ON analogs of nucleic acids. Various side-chain linkers enhancing ability of the -deoxyadenosine strand to create a double helical complex with the target DNA or RNA strand were tested. Linkers were based on either lysine (butyl-amino phosphoramidates) or arginine (butyl guanidinium phoshoroamidates) side chains (naturally occurring in proteins). Several double helical structures (parallel oriented) consisting of the natural chain (either dT_{12} or rU_{12}) and cationic -deoxyadenosine counterpart were used as model systems. Fully solvated molecular dynamics simulations revealed that both types of tethers are able to bridge the minor groove (slightly narrowed in the case of atypical parallel oriented hybrid duplexes) and interact efficiently with phosphate groups in the complementary natural strand. Direct hydrogen bonds between positively charged heads of linkers and negatively charged phosphate groups were established within the MD runs. Average values as well as time-development of distances between groups participating in the aminoalkyl-phosphate hydrogen bonding were analyzed in details. Differences between -DNA:DNA and DNA:RNA systems (as a consequence of various minor groove width) were found.

This work was supported by the Grant of the Ministry of Education, Youth and Sports of the Czech Republic (project No. MSM 0021620835) and the Grant Agency of Czech Republic (project No. 203/05/0827 and No. 202/02/D114). Results have been partially obtained using computer facilities of the Metacentrum of the Czech Universities.

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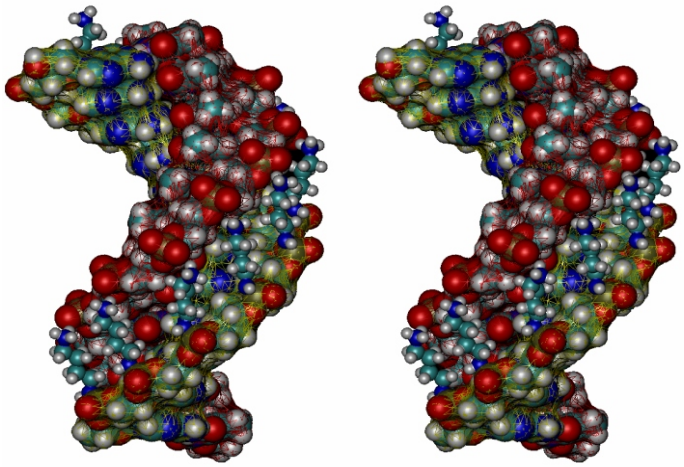

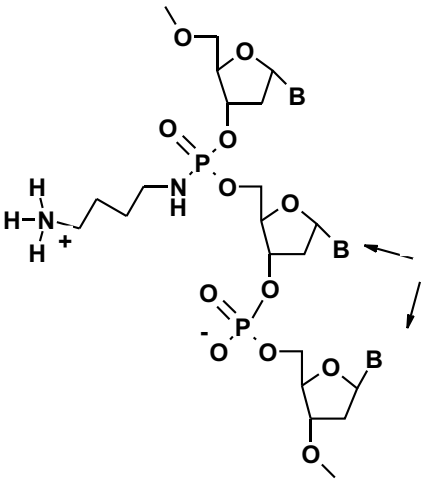

P2

MOLECULAR DETERMINANTS OF THE AGONIST BINDING SITE OF HUMAN MT2 MELATONIN RECEPTOR

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The hormone melatonin is known to play an important role in the regulation of physiological and neuroendocrine functions [1,2]. To better understand the mechanism of interactions between G protein-coupled melatonin receptors and their ligands we have generated a homology model of the helical part of the human MT2 melatonin (hMT2) receptor using the structure of bovine rhodopsin as a template [3]. Molecular modeling has been combined with site-directed mutagenesis to investigate the role of the specific amino acid residues within the transmembrane domains (TM) number 3, 5, 6 and 7 of hMT2 receptor in the interaction with 2-iodomelatonin. Selected residues were mutated and radioligand binding assay was used to test the binding affinities of hMT2 receptors transiently expressed in HEK293 cells. Our data demonstrates that residues N268 (N6.52) and A275 (A6.59) in TM6 as well as residues V291 (V7.36) and L295 (L7.40) in TM7 are essential for 2-iodomelatonin binding to the hMT2 receptor, while TM3 residues M120 (M3.32), G121 (G3.33), V124 (V3.36) and I125 (I3.37) may participate in binding of other receptor agonists and/or antagonists.

This work was supported by Grants 309/02/1479, 309/04/0496, and 204/03/0714 of the Grant Agency of the Czech Republic; by Grant B5011308 of the Grant Agency of the Czech Academy of Sciences; by Research Projects 1K03020 and MSM 1131 00001 of the Ministry of Education, Youth and Sports of the Czech Republic, and by Research Project AVOZ 5011922.

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P3

INFLUENCE OF PYRENE ON THE STRUCTURE AND DYNAMICS OF DPPC BILAYER

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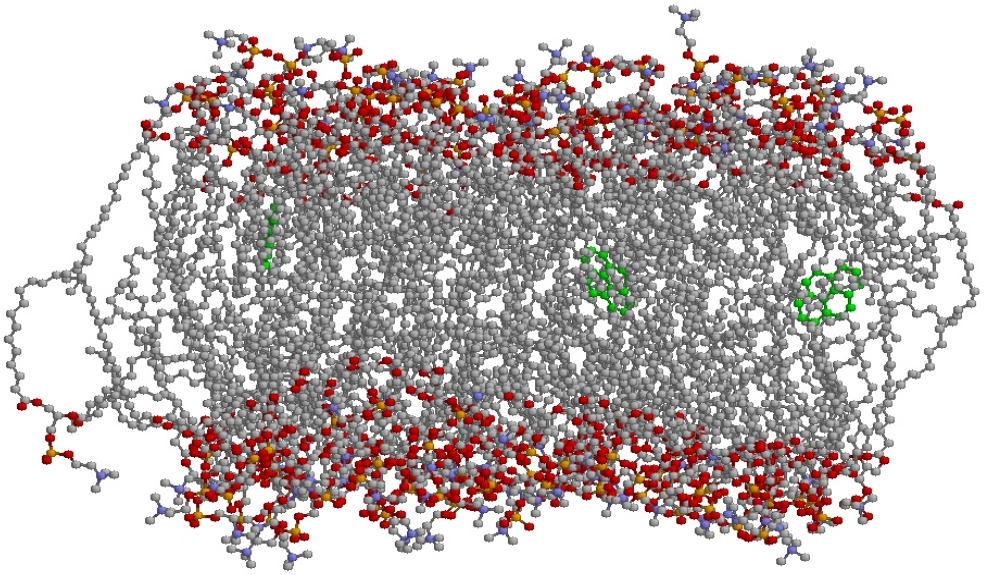
We have employed 20 ns molecular dynamics simulation to study the influence of pyrene probe on structure and dynamics of dipalmitoylphosphatidylcholine (DPPC) bilayer. Pyrene is an important fluorescent probe used for monitoring excimer fluorescence. Our simulated system contains 128 molecules of DPPC, 3655 water molecules and various concentrations of pyrene. The simulations were performed using the Gromacs package [1].

Concentrations of pyrene in lipid membrane were 0:128 and 3:128 at different temperatures. We have chosen two temperatures below the main phase transition of DPPC (314,15 K) and two above.

From our analysis of the trajectory we have obtained information about structural changes caused by presence of pyrene probe in the membrane. Molecular dynamic simulation has shown that the pyrene probe is located in the acyl chains region of DPPC. The major influence of the probe on the lipid membrane is in the nearest surroundings of pyrene. On the other hand the influence of pyrene in the membrane-water interface is negligible.

We have found out decreases thickness of the DPPC membrane with increasing temperature. The membrane with pyrene probes is thicker than in the case without pyrene. The thickness of DPPC membrane without pyrene has decreased noticeably between the temperature of 310 K and 325 K which corresponds to main phase transition of DPPC. In the case of membrane with pyrene we have observed similar decrease of thickness already between 293 K and 310 K. These results probably indicate the decrease of temperature of the main phase transition due to the presence of pyrene in the membrane.



to the abstract by J. Čurdová

P4

PROTEINS OF OXYGEN EVOLVING COMPLEXES OF PHOTOSYSTEM II STUDIED BY MEANS OF MOLECULAR MODELING AND VIBRATIONAL SPECTROSCOPY

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Homology and energetic modeling is used for the determination of the structure of several proteins of the oxygen evolving complex (PsbQ, PsbP and PsbO) bound to photosystem II of higher plants, spinach and pea. Structural and sequence alignment of the individual proteins of the oxygen evolving complex was performed to get a basis for homology modeling. Three-dimensional models of oxygen evolving complex proteins are suggested. These models will serve in future to get detailed information about the secondary structure content, the spatial arrangement and interactions of individual aromatic residues that can be confronted with results from vibrational spectroscopy. Spectroscopic data will serve as a feedback for the generation of three-dimensional models. Thus gained models will be used for the detection of interaction sites not only by Raman and FTIR, but will also be used for fitting into images of the whole complex of photosystem II taken by electron microscopy. The work presented here is the first step to determine the spatial arrangement of individual proteins in the oxygen evolving complex.

Supports from the Institutional Research Concept of the Academy of Science of the Czech Republic (No. AVOZ60870520) and from the Ministry of Education of the Czech Republic (No. MSM6007665808) and the Grant Agency of the Czech Republic (grant No. 206/03/D082) are gratefully acknowledged.

P5

STRUCTURE AND DYNAMICS OF THE -*N*-ACETYLHEXOSAMINIDASE IN NATIVE AND DEGLYCOSYLED FORM REVEALED BY HOMOLOGY MODELING AND VIBRATIONAL SPECTROSCOPY

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Glycoprotein -*N*-acetylhexosaminidase from *Aspergillus oryzae* catalyses hydrolysis of chitobiose into constituent monosaccharides. The enzyme is physiologically important during the life cycle of fungi [1]. There is an interest in the catalytic mechanism by which these enzymes cleave their substrate since these processes are important in human diseases and control of fungal and insect pests.

Homology modeling with the Modeller program [2] used the coordinates for a *S. marcescens, S. plicatus* and human lysosomal-hexosaminidase (enzymes of glycohydrolase family 20 with a 44% degree of similarity). Refinement was achieved through algorithmic analysis and minimization with the TRIPOS force field in the SYBYL/MAXIMIN2 module. The model structure has been confronted with data from FTIR and Raman spectroscopy obtained with the enzyme (both native and deglycosylated form) purified from the medium of the producing organism *A. oryzae*. The secondary structure determined from analysis of FTIR amide I and II bands and Raman amide I corresponds well to our molecular model. The results from Raman and FTIR spectroscopy demonstrate the differences in secondary structure content caused by deglycosylation of the protein.

Thermal dynamics in the range of 5–70 °C monitored by Raman spectroscopy and analyzed by homospectral 2D-correlation analysis revealed also significant changes in dynamics of proteins in native and deglycosyled forms. Glycans attached on the surface of the protein stabilize mostly hydrophobic parts of -sheet structures that can prevent the proteins from aggregation. Therefore, we can conclude that our model of -*N*-acetylhexosaminidase brings better understanding of the protein structure and dynamics with respect to its carbohydrate part.

Support from the Institutional Research Concept of the Academy of Science of the Czech Republic (No. AVOZ50200510, No. AVOZ60870520) and from the Ministry of Education of the Czech Republic (No. MSM0021620835, No. MSM6007665808) and the Grant Agency of the Czech Republic (No. 203/04/1045) is gratefully acknowledged.

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P6

SMALL ALTERATIONS IN 3D STUCTURE OF A PROTEIN CAN ANSWER FOR IMPORTANT FUNCTIONAL DIFFERENCES – EF-TU STORY

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Protein synthesis elongation factor Tu (EF-Tu) represents one of the major components of translation system in prokaryotes. It participates on the correct positioning of the incoming aminoacyl-tRNA on the ribosome where polypeptide chain is synthesised. Decoding of the information in mRNA via codon/anticodon interaction is mediated by ternary complex consisting of EF-Tu GTP.aminoacyl-tRNA. Repetitive participation of EF-Tu in elongation cycle requires its interaction with a number of ligands, among them the most important are guanine nucleotides GDP and GTP. The protein is represented by three-domain structure and behaves like a typical G (guanine nucleotide binding) protein. Interaction of flexible domain 1 containing GDP/GTP binding pocket with more rigid domains 2 and 3 allows it to work as a molecular switch changing between "on" and "off" conformation upon binding of GDP or GTP. There are available specific inhibitors of EF-Tu, which are able to "freeze "the protein in either "on", or "off" conformation, as an example can be mentioned kirromycin or pulvomycin. The protein is recognized as a classical cyto-

plasmic protein, however, thanks to some of its below listed features, it may be considered as a special case. In some organisms it has been assigned for very special functions, although the primary sequence of the protein is highly conserved through out prokaryotes.

Besides the role in translation, EF-Tu is proposed to function in other compartments of the cell metabolism and that may be the reason, why this protein is a subject of a number of post-translation modifications. Some of them are playing the role in translation, others are important for its potential functions outside of the elongation cycle. In *E. coli*, *Bacillus subtilis* and *Bacillus licheniformis* a part of EF-Tu population, which is located on the membrane, can be methylated in response to starvation for an essential nutrient.

EF-Tu from *E. coli* and *T. thermophilus* was found to be phosphorylated *in vivo*, and the phosphorylated fraction remained stable under different conditions. Since the phosphorylated residue (Thr-382) is conserved in all known EF-Tu corresponding sequences from other species, the phosphorylation might be a common phenomenon. During last few years this protein was found to function as an adhesion factor, for instance EF-Tu of *M. pneumoniae* binds fibronectin as part of a virulence mechanism. Another example is EF-Tu of *Lactobacillus johnsonii*, which mediates attachment of these bacteria to intestinal epithelial cells and mucins and stimulates proinflammatory reactions.

We described previously a spontaneous polymerisation of EF-Tu from *Streptomyces aureofaciens,* which might serve as a protective mechanism for EF-Tu present in spores or enables the protein to play a structural role. Aggregates are formed under physiological conditions and give raise to filamentous structures large enough to be visible in the light microscope. We have developed simple and effective method for purification of large amounts of the aggregated protein, which retains its nucleotide binding activity. We have found that two closely related strains of *Streptomyces aureofaciens* contain EF-Tu capable of spontaneous aggregation in contrast to number of other *Streptomyces* species of which EF-Tu gene was cloned and protein isolated. We purified EF-Tu from both strains using method mentioned above and performed on them comparative studies in order to understand better the structural and functional features of this phenomenon. Using 2D electrophoresis of purified proteins and their hydrolysis products we analysed their structural differences and heterogeneity resulting from their post-translation modifications. We sequenced *tuf* genes coding EF-Tu in both strains and performed comparative 3D modelling of their structures and those of other *Streptomyces* strains with emphasis on their surface structures.

P7

DETERMINATION OF STRUCTURE AND FUNCTIONAL PROPERTIES OF CYTOPLASMIC TERMINUS OF VANILLOID RECEPTOR TRPV1

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The vanilloid receptor TRPV1, a member of TRP channel family, has function as a multimodal signal transducter of noxious stimuli in the mammalian somatosensory system [1]. The TRPV1 is consisted of six transmembarane-spanning domains with a pore forming region between fifth and sixth domains, and cytoplasmically located C- and N-terminal regions. Although structural and functional studies have been done [2, 3], the possible contributions of terminal regions to vanilloid receptor function remain elusive. To determine structure and functional properties of the cytoplasmically located tails, the DNA fragments encoding for the N- and C- terminus were cloned to the expression vectors and transformed to *E. coli* strain. Overexpressed proteins were purified by affinity chromatography and used for structural analysis by a wide range of low resolution methods. Experimental results were combined with homology and energetic modeling techniques and we propose a three-dimensional structure of the C-terminus.

This research was supported by the Ministry of Education, Youth and Sports of the Czech Republic (MSM60076658 08) and by the Academy of Sciences of the Czech Republic (Institutional research concepts AVOZ50110509 and AVOZ60870520)

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P8

MONITORING OF ZWITTERIONIC PROLINE AND ALANINE CONFORMATIONAL SPACE BY RAMAN OPTICAL ACTIVITY

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Raman optical activity (ROA) measures vibrational optical activity by means of a small difference in the intensity of Raman scattering from chiral molecules in right and left circularly polarized incident laser light. The ROA spectra of a wide range of biomolecules in aqueous solutions can be measured routinely. Because of its sensitivity to the chiral elements, ROA provides new information about solution structure and dynamics, complementary to that supplied by conventional spectroscopic techniques [1].

Natural targets for ROA technique are amino acids and peptides. However, the interpretation of the spectra is almost entirely dependent on *ab-initio* modeling of vibrational frequencies and spectral intensities, which imposes limits on molecular size and overall accuracy.

Incident circular polarization (ICP) ROA instrument has been built at the Institute of Physics following the design of the instrument constructed in Glasgow [2]. Combination of experimental and computational approaches represents unique and powerful tool for studying structure and interactions of biologically important molecules.

Computation of ROA is a complex process, including evaluation of equilibrium geometry, molecular force fields and polarizability tensor derivatives. In case of zwitterionic amino acids and peptides many complications arise also from their conformational flexibility and strong interaction with the solvent, which has to be taken into account in the modeling. For our ROA simulations we used continuum solvent models and solvation with explicit molecules of water [3].

Conformational space of L-alanine was investigated in detail by rotating the NH_3^+, CH_3 and COO^- groups. Our calculations suggest that NH_3^+ group is freely rotating while CH_3 and COO^- groups rotate only limitedly. Proline molecule contains a non-planar five-member ring and exhibits two major conformations with very similar energies. Conformational space of L-proline was examined by puckering the ring and also rotating COO^- group. Weighted average spectra that were constructed can explain natural broadening of several spectral bands in particular in the low wavenumber region.

Finally we have shown that the simulation techniques requiring consideration of system dynamics and averaging over molecular conformations and solvent configurations are able to provide realistic ROA spectra of flexible and polar molecules.

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P9

CRYSTALLIZATION OF PHOTOSYNTHETIC PROTEINS FROM *PISUM SATIVUM* – A NEW CRYSTALLIZATION STRATEGY

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The photosystem II (PSII) is a multisubunit membrane-protein complex consisting of membrane in-lying subunits, hydrophilic peripheral subunits and large number of cofactors, including chlorophylls, pheophytins, carothenoids, plastoquinones, iron and manganese, which together trap, transfer and modulate a solar energy to drive catalytic photoinduced oxidation of water and synthesize molecular oxygen. Catalytic mechanism of PSII has been studied using a wide range of approaches [1-3], but particular molecular details of water oxidation catalyzed by the oxygen evolving center (OEC) remains unclear. Crystallographic studies of OEC PSII from thermophilic cyanobacterium [4-6] have given the first description of the structure of PSII, but these models are not absolutely complete as yet.

Finding suitable crystallization condition is the main problem to solve a protein structure by X-ray diffraction techniques. The current crystallization strategies are mainly based on the screening upon previously successful chemical cocktails and on classical crystallization techniques based on evaporation. That strategy has been found partially successful for soluble globular proteins. However, membrane protein is a particular case for which the success rate applying commercial crystallization screens is much lower than for soluble proteins. The fact that membrane proteins are often unstable, highly temperature and light sensitive together with their complicated composition are responsible for difficult crystal growing and solving their structure.

Here we report a new approach for crystallization of monomeric photosystem II core complex (OEC PSII) from green pea. The core complex of PSII was isolated from *Pisum sativum*, purified and concentrated to 2-3 mg/ml of chlorophyll *a* (10-15 mg/ml of protein). The protein solution was prepared containing additives 10mM NaCl and 1mM $MnCl_2$ used in crystallization trials [7]. Freshly isolated and frozen samples of PS II protein complex were crystallized using the counter-diffusion technique implemented in single capillaries [8] and traditional vapor diffusion method in sitting drops. After performing of several series of crystallization experiments it was found that only fresh purified and non-frozen protein is suitable for crystallization trials. Crystallization in capillaries was ascertained as an efficient method to find and optimize crystallization conditions.

The core complex of PSII crystallized in green needle-shaped crystal form from precipitant solution containing PEG4000 and MPD in MES pH 6.50 at 291-293K. Protein character of PSII crystals was confirmed by laser spectroscopy under use of Olympus IX70 inverted microscope (Olympus, Japan) equipped with Triax320 monochromator and CCD camera (Jobin Yvon, France), and by X-ray diffraction measurements. Frozen monocrystals of PSII core complex were tested at the synchrotrons DESY, beamline X13 (Hamburg, Germany) and ESRF, beamlines BM16 and ID14-1 (Grenoble, France) and at the home source diffractometer at IMG AS CR (Prague, CZ). Diffractions at low resolution have also certified protein character of crystals. As the crystals were very small and insufficiently ordered for high resolution analysis, further experiments will be aimed at obtaining better-quality crystals from which the structure of PSII complex of higher plant could be solve.

This work is supported by the Grant Agency of the Czech Republic (grant 206/03/D061), by the joint C.S.I.C. and AS CR project 2004CZ0003 in the frame of the cooperation agreement P2004CZ01, by the Ministry of Education of the Czech Republic (MSM6007665808) and by the Academy of Sciences of the Czech Republic (Institutional research concept AVOZ60870520).

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P10

THE BINDING OF SELECTED LIGANDS TO HUMAN $_1$-ACID GLYCOPROTEIN STUDIED BY COMBINED APPROACH OF MOLECULAR MODELING AND VIBRATIONAL SPECTROSCOPY

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Human $_1$-acid glycoprotein (AGP), also known as orosomucoid, is a 41-kDa single polypeptide consisting of 183 amino acids. It contains 42% carbohydrate in weight and has up to 16 sialic acid residues. AGP, a human blood plasma protein, belongs to the lipocalin family of proteins, a heterogeneous group of proteins that bind a variety of small hydrophobic ligands. It is known that AGP plays a role under inflammatory or other pathophysiological conditions and is able to bind basic drugs and certain steroid hormones such as progesterone. However, its biological function and 3D structure remains unknown [1].

Three-dimensional structure of AGP has been predicted recently [2] by an approach that combines molecular modeling and vibrational spectroscopy [3]. The model shows that AGP folds as a highly symmetrical all- protein dominated by a single eight-stranded antiparallel -sheet. The Raman difference spectroscopy confirmed docking predicted binding pocket for progesterone [2].

Here we present critical assessment of binding of several ligands with different physico-chemical properties to AGP (e.g. progesterone, propranolol, warfarin). In silico docking of ligands into the binding pocket of our model was explored with AutoDock program [4] and followed by molecular dynamics studies in Gromacs software package. In vitro binding of ligands was studied by means of Raman difference spectroscopy that clearly confirmed presence of Trp^{122} in the binding pocket for all the ligands. It supports computed results that AGP has only one binding site for presented ligands. Thermal dynamics in the range of 20–70 °C monitored by Raman spectroscopy and Fourier-transform infrared spectroscopy and analyzed by homo- and hetero-spectral 2D-correlation analysis [5] revealed full reversibility of the protein motion upon heating dominated by thermal "breathing" of the -barrel which points at high stability of protein itself but mostly at stability of binding pocket which is localized insight the -barrel.

Ministry of Education of the Czech Republic (No. MSM 0021620835, No. MSM6007665808) and the Academy of Science of the Czech Republic (No. AVOZ60870520) are gratefully acknowledged for support.

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P11

NMR DISTANCE RESTRAINTS AT LOW TEMPERATURES: AN APPLICATION TO DNA HAIRPIN

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Number of the experimental NMR distance restraints extracted at the ambient temperature is remarkably decreased by an internal mobility in a molecule, mostly chemical exchange. This problem can be avoided by NMR measurements at lower temperatures. To achieve the lowest temperature while keeping the liquid state and minimize the chemical exchange, the NMR experiments can be performed using the slowly cooled capillaries [1].

The aim of the present study is to investigate possibilities of NMR measurements in liquid state at the temperatures below 273 K and applicability of this approach to a nucleic acid fragment. For this purpose, the d(GCGAAGC) hairpin has been chosen because its structure has been previously solved precisely by NMR spectroscopy [2]. We have performed a new structure calculation of this molecule using the low temperature NMR measurements and compared the precision of the newly calculated and known structures.

A series of the 2D-NOESY spectra with the mixing times in a range of 50 to 400 ms has been measured at 268 K. The NOESY cross-peaks for the different mixing times were integrated using the SPARKY program and the results were employed to derive the inter-proton distance restraints. Then, the numbers of distance restraints extracted at 268 K and 303 K were compared. In case of non-exchangeable protons, these numbers were comparable due to the significant spectral overlaps in the NOESY spectra measured at 268 K. While, there was almost twice higher number of exchangeable proton distances extracted at 268 K compared to 303 K and these restraints helped increase the precision of the calculated structure of the d(GCGAAGC).

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P12

THEORETICAL STUDY ON THE WATER AUTODISSOCIATION PROCESS IN SMALL CLUSTERS

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Despite large progress in computational facilities, obtaining of reliable hydration free energies still presents a difficult task. In this work, a model of hydration based on explicit treatment of water molecules was investigated. Product state of the autodissociation process – the ion-pair formation was modeled in small water clusters (up to 21 monomers) using correlated ab-initio methods. The influence of cluster size and geometry on the resulting pKa (pH) value was examined. The pH values decrease with increasing cluster size going from pH~25 in the dimer case to pH~5 for hexamer. For the correct pH determination, the Boltzmann weighting of all the assumed clusters has to be considered. Topological arrangement of H-bonds rather then cluster size determines the pH values of larger clusters. The pH values range from 6 to 10.

Calculations based on the largest 21-water structure indicate that the ion-pair formation inside the cluster requires significantly higher energy compared to the ion formation on the cluster surface.

It was also found a correlation between the distance between the monomers involved in proton transfer and the free energy of the autodissociation process. This enables us to predict (or estimate) the pH values without very demanding calculations.

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14-3-3 C-TERMINAL STRETCH CHANGES ITS CONFORMATION UPON LIGAND BINDING AND PHOSPHORYLATION AT THR232

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14-3-3 proteins are abundant binding proteins involved in many biologically important processes [1,2]. 14-3-3 proteins bind to other proteins in a phosphorylation-dependent manner and function as scaffold molecules modulating the activity of their binding partners. In this work, we studied the conformational changes of 14-3-3 C-terminal stretch, a region implicated in playing a role in the regulation of 14-3-3 [3]. Time-resolved fluorescence and molecular dynamics have been used to investigate structural changes of the C-terminal stretch induced by phosphopeptide binding and phosphorylation at T232, a casein kinase I phosphorylation site located within this region. Tryptophan residue placed at position 242 has been exploited as an intrinsic fluorescence probe of the C-terminal stretch dynamics. Other tryptophan residues were mutated to phenylalanine. Time-resolved fluorescence measurements revealed that phosphopeptide binding changes the conformation and increases the flexibility of 14-3-3 C-terminal stretch, demonstrating that this region is directly involved in ligand binding. Phosphorylation of 14-3-3 at T232 resulted in inhibition of phosphopeptide binding and supression of 14-3-3-mediated enhancement of serotonin N-acetyltransferase activity. Time-resolved fluorescence of W242 also revealed that phosphorylation at T232 induces significant changes of the C-terminal stretch conformation. In addition, molecular dynamics simulations suggest that phosphorylation at T232 induces more extended conformation of 14-3-3 C-terminal stretch and changes its interaction with the rest of the 14-3-3 molecule. These results indicate that conformation of the C-terminal stretch plays an important role in the regulation of 14-3-3 binding properties [4].

This work was supported by Grants 204/03/0714 and 309/02/1479 of the Grant Agency of the Czech Republic; by Grant B5011308 of the Grant Agency of the Czech Academy of Sciences; by Research Projects 1K03020, MSM 1131 00001, and 1132 00001 of the Ministry of Education, Youth and Sports of the Czech Republic, and by Research Project AVOZ 5011922.

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P14

THE ROLE OF PIGMENTS OF PHOTOSYSTEM II REACTION CENTER, A COMPUTATIONAL STUDY

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Photosystem II (PSII) is a pigment-protein complex located in thylakoid membrane of cyanobacteria, algae and higher plants. It performs series of light driven reactions, which result in a separation of charge and subsequently in a reduction of an electron-transport chain and water oxidation. Primary site of the light to chemical energy conversion is located in so-called reaction center (RC). Recently, light induced absorption and circular dichroism spectra from PSII RC, in presence of artificial electron donor dithionite, were obtained [1]. At room temperature the reduced pheophytin *a* (PHO) induces a conformational changes of the RC protein environment which affects the excitonic interaction of the RC chlorophylls.

Classical simulation methods such as molecular dynamics (MD) of PSII RC could be a powerful tool for better understanding and interpreting mention spectra. First, the distribution of negative charge over the molecule of PHO alone was needed to be calculated. Charges were derived by fitting HF/6-31G* *ab initio* electrostatic potential on a set of grid points around the molecule and subsequently

rescaling them according to the restrained ESP (RESP) methodology [2] widely applied in AMBER Force Field (FF) [3]. Similarly also Mulliken charges applied in CHARMM FF [4] were computed. Due to computational demanding size of the whole PS II complex found in recent experimental crystal structure from *Thermosynechococcus elongatus* (PDB code: 1S5L) [5], smaller-size system including the most important parts of PSII RC was constructed.

Various studies have been recently performed in order to collect a complete set of parameters for the cofactors of PS II RC. [6, 7, 8, 9, 10]. Different methodologies were used to obtain FF parameters for the cofactors of PSII complex, namely fitting *ab initio* calculated data to observed vibrational frequencies of nickel octamethylporphyrin [9], performing normal mode analyses based on vibrational spectra of model compounds [9], using *ab initio* methods based on the density functional theory (DFT) [11]. It has been found that the current CHARMM27 FF parameters for the reduced heme group without ligands could represent the geometries obtained by QM calculations but are in partial disagreement with spectroscopic data [12, 13].

In this work we primarily focus on obtaining a charge set for both charged and neutral forms of PHO as well as on developing new CHARMM FF parameters [6]. The departure points of our force field development have been the existing CHARMM27 FF potential parameters for the unligated reduced form of heme prosthetic group [9] and quinines incorporated in the PSII RC.

The most characteristic feature of chlorophylls and pheophytins is their tertapyrrolic architecture related directly to metalloporphyrin (a magnesium-porphyrin system). Unfortunately, the PSII RC cofactors are in part composed of some unique molecular fragments not included in the present distributions of CHARMM27 FF [4], AMBER99 FF [3] and other [7, 6, 8] databases for which an *ex novo ab initio*-based modelization was performed. Following the scheme detail depicted in reference [7] we started to develop FF parameters (compatible with the CHARMM27 FF) for PSII RC cofactors based on a reliable set of *ab initio* calculations and reference data for chlorophyll *a*, pheophytin *a*, and a derivate of ubiquinone. In building the FF parameters we have limited the new types of atoms added to CHARMM27 FF to the bare minimum. In this study we only present the bond stretching parameters, the equilibrium bond lengths and subsistent force constants. The rest of parameters for bonded interactions are in progress.

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P15

PROGRESS IN AUTOMATIC BIOMACROMOLECULAR MODEL BUILDING

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Automatic model building is an open challenge in protein crystallography. Three principally different approaches have been developed so far in various protein laboratories: (i) ARP method (based on an interpretation of the electron density in terms of oxygen globs iteratively followed by atomic refinement). (ii) Skeltonization. (iii) Positioning of rigid protein fragments in the electron density by graphical or FFT methods.

Accurate automatic protein model building, based on an expansion of the electron density in spherical harmonics and spherical Bessel functions, phased rotational and translational function and on flexible fragment concept, has been described in a series of papers [1-3] by author. The protein model is built with an accuracy of about 0.2 Å at resolutions of 1.2-1.9 Å. Partial results (e.g. 90% of polyalanine structure) can be obtained at resolutions 2.0-2.3 Å. The recent advances in the method development and computer program will be presented.

Phased rotational, translational and conformation function.
The phased rotational and translational function has been generalized from six-dimensional to multidimensional function. Conformational search was added. Instead of using different fragments for each conformer only one fragment is used and required conformation is calculated on fly. Only the best conformer is stored at particular position and refined.

Loop building.
Hydrophilic residues on the protein surface are usually part of the loop structure. Residues forming loops are sometimes disordered or having large thermal movements. The electron density at loop position is weak, difficult to interpret and in many cases confused with hydrogen-bonded water molecules. If sequence is correctly assigned to the main-chain, number of missing residues in known ad the loop can be constructed. In the method proposed, the both ends of two unconnected chains are extended randomly with AlphA0 fragments. Three factors are then optimized: overlap (mean distance) of peptide groups, electron density fit and Ramachandran energy. Ramachandran energy (2-D periodic function) is expressed as a set of Fourier coefficients. Fourier coefficients are calculated by Fourier summation from pseudo-atomic representation. The minima on Ramachandran map are modeled by different atoms and the curvature of the minimum by a temperature factor.

Least-square refinement of the group model.
Standard nonlinear least-squares were applied to protein refinement. Each group (fragment) is treated as a generalized atom. The number of parameter to be refined is reduced approximately by a factor of four compared to atomic refinement. The method is more-or-less equivalent to a rigid body refinement. The only difference is that in the group model the groups are overlapped.

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P16

DFT THEORETICAL DESCRIPTION OF Cu(I)/Cu(II) INTERACTIONS WITH VARIOUS LIGAND FIELDS

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Both monovalent and divalent copper cations are essential for an occurrence of many processes in bioorganisms. They are present in active centers in number of redox enzymes, for example in so called blue proteins. Hence, quantum chemical investigation of copper interaction with key ligands is useful and important. This presentation summarizes our studies of Cu^{+} and Cu^{2+} cations in variable sulphur-ammonium-water ligand fields. At first, structures were optimized at DFT level using B3PW91 functional. Double-basis set with additional diffusion and polarization functions was used. Inner electrons of Cu and S atoms were described by effective pseudopotentials. Stabilization energies and other electronic properties were calculated with B3LYP functional with more accurate augmented triple-zeta basis set.

It was found that the optimal Cu(I) coordination involves two directly bonded solvent molecules for ammonium-water ligand field. If SH_2 ligand molecules are

present, Cu^{+} cation tends to 3 or even 4 coordination. The Cu^{2+} cation prefers four and five coordinated complexes for all types of ligand fields. If strength of metal-ligand dative bonds is compared, the following order would be predicted: ammonium, water, and sulphur. Therefore NH_3 molecules always stay in the first solvation shell in mixed compounds. Our studies also contained Natural Population Analysis that gives further support of the calculated results and enables detailed insight to the calculated systems. The most stable structures correlate with the highest donations of ligand electron densities to copper atom.

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NMR STUDY OF PROTEIN POTENTIALLY INVOLVED IN MOLYBDOPTERIN SYNTHESIS

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Molecule of interest, TA1019, is a protein possibly involved in molybdopterin synthesis. It consists of 107 amino acid residues. Structural study of TA1019 represents a part of proteomic project of a prokaryote organism, *Thermoplasma acidophilum*. The recombinant protein TA1019 was prepared at the University of Toronto, Canada, by Adelinda Yee and coworkers. Aggregation and low stability of the sample excluded this project from automatically performed structural investigation. Due to this fact, the sample was offered to our laboratory and subjected to careful structure elucidation by NMR. At the present time, were made first basic steps in the course of structural determination, i.e., assignment of the measured frequencies to the individual atoms of the protein molecule. The obtained backbone sequential assignment and partial side chain assignment is a necessary prerequisite for 3D structure determination. In addition, knowledge of resonance frequencies of certain nuclei permits preliminary secondary structure prediction.

P18

CHARACTERIZATION AND CRYSTALLISATION OF DIVIVA MUTANT PROTEINS

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Gram-positive bacterium *Bacillus subtilis* is the one of the most commonly studied model organism. Its life cycle represents a complex biological process. The mechanisms that ensure the correct positioning of the septum are complicated and still intensively studied. A machinery of proteins is involved.

DivIVA protein is a bifunctional protein [1]. During vegetative growth it acts as a controller of the mid-cell division site positioning. There is an inhibition complex of two proteins: MinC and MinD, which disable assembly of division machinery (first and foremost assembly of FtsZ ring), in positions where it occurs. A DivIVA protein arrests MinCD inhibition complex at the cell poles and thus releases the cell center for septum formation [1, 2, 3, 4]. During sporulation DivIVA is involved in chromosome segregation [1].

DivIVA is a 19,5 kDa tropomyosin-like -helical coiled-coil protein. Cryonegative stain transmission electron microscopy revealed 3D structure of DivIVA consisting of particles with lateral expansions at both ends resembling a “doggy-bone”. These forms are about 145 kDa, which correspond to 6-8 mers. DivIVA oligomers can build higher structures like strings, wires and 2D nets [5].

It was found, that DivIVA amino acid residues 17, 18 and 19 are responsible for polar targeting of protein. So mutations at these sites may mimic conformational change that probably occurs and enables DivIVA its dual function [6]. We have cloned the *divIVA* localization mutant genes into expression vectors to prepare proteins for cryonegative stain transmission electron microscopy and for crystallization trials.

Work in author’s laboratory is supported by grant 2/1004/22 from the Slovak Academy of Sciences and Wellcome Trust Project and Collaborative Research Initiative Grants 066732/Z/01/Z.

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P19

LYMPHOCYTE ACTIVATION RECEPTORS: NEW STRUCTURAL PARADIGMS IN THE GROUP V OF C-TYPE ANIMAL LECTINS

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From the point of view of structural biology, our understanding of the structure – function relationships in the C-type lectin family remains fragmentary. By far the best characterized molecule of the whole family is the soluble mannose binding protein studied protein crystallography. This protein binds to calcium ions in the ligand-binding domain, and the binding of calcium is intimately linked to the recognition of carbohydrates. Some of the receptors of natural killer lymphocytes have been also structurally characterized. Since this group is evolutionarily most divergent, interesting structural paradigms have been observed with regard to binding of calcium, carbohydrates, and other ligands. In NKR-P1, calcxium may not be easily removed by chelating agents because of its unique chemical nature. In CD69, binding of calcium causes a structural shift in amino acids important for binding of carbohydrates. Structural studies have also allowed us to understand an interesting preference of these receptors for either linear (NKR-P1) or branched (CD69) carbohydrate sequences.sugars. Remodeling of the binding surface in CD94 or Ly-49 opens the way for specific recognition of protein and peptide ligands.

This work was supported by Ministry of Education of the Czech Republic No. MSM 0021620808, by Institutional Research Concept No. AVOZ 50200510, by Grant Agency of the Czech Republic No. 301/05/P567, and by Grant Agency of the Academy of Sciences of the Czech Republic No. A5020403.

P20

RIBOSOMAL RNA KINK-TURN MOTIF – A FLEXIBLE MOLECULAR HINGE

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Ribosomal RNA K-turn motifs are asymmetric internal loops characterized by a sharp bend in the phosphodiester backbone resulting in "V" shaped structures, recurrently observed in ribosomes and showing high degree of sequence conservation. We have carried out extended explicit solvent molecular dynamics simulations of selected K-turns, in order to investigate their intrinsic structural and dynamical properties. The simulations reveal an unprecedented dynamical flexibility of the K-turns around their X-ray geometries. The K-turns sample, on the nanosecond timescale, different conformational substates. The overall behaviour of the simulations suggests that the sampled geometries are essentially isoenergetic and separated by minimal energy barriers. The nanosecond dynamics of isolated K-turns can be qualitatively considered as motion of two rigid helix stems controlled by a very flexible internal loop which then leads to substantial hinge-like motions between the two stems. This internal dynamics of K-turns is strikingly different for example from the bacterial 5S rRNA Loop E motif or BWYV frameshifting pseudoknot which appear to be rigid in the same type of simulations. Bistability and flexibility of K-turns was also suggested by several recent biochemical studies. Although the results of MD simulations should be considered as a qualitative picture of the K-turn dynamics due to force field and sampling limitations, the main advantage of the MD technique is it ability to investigate the region immediately around their ribosomal-like geometries. This part of the conformational space is not well characterised by the solution experiments due to large-scale conformational changes seen in the experiments. We suggest that K-turns are well suited to act as flexible structural elements of ribosomal RNA. They can for example be involved in mediation of large–scale motions or they can allow a smooth assembling of the other parts of the ribosome.

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STRUCTURAL MEASUREMENTS ON MEMBRANE PSBH PROTEIN IN DIFFERENT LIPID/-DETERGENT ENVIRONMENTS

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One of the key components for the assembly of Photosystem II is the psbH protein [1]. PsbH is one of the proteins expressed in etiolated and illuminated leaves on the same level in higher plants, which indicates that its function may be considered separately from the rest of the multiprotein complex.

The PsbH protein of cyanobacterium Synechocystis sp. PCC 6803 was expressed as a fusion protein with glutathione-S transferase (GST) in E. coli [2]. We isolated the 15N labeled PsbH protein in concentration of 1.1 mg/ml in presence of detergent octyl glucoside (OG). We also isolated non-labeled protein for preliminary lipid titration experiments measured by circular dichroism (CD) spectrometer. Molecular Dynamics experiments on a homolgy model of the PsbH protein were carried out to compare the secondary structure changes with the results from CD.

The liposomes were prepared by reverse-phase evaporation technique from the thylakoid membrane lipids; sulphoquinovosyl diaglyceride (SQDG), digalactosyl diglyceride (DGDG), monogalactosyl diglyceride (MGDG) and phosphatidyl glycerol (PG). The most favourable lipid, which induced complex protein folding, detected as formation of the negative band approx. 222 nm in CD spectra, seemed to be PG. Very similar changes were observed at higher concentration also in SQDG, however folding of clearly different nature was achieved upon titration by DGDG. This indicates that the protein folding may not be directly related to specific binding of lipids, rather we observe two different types of folding in lipid bilayers of two different properties.

The CD measurements revealed folding of the PsbH protein in detergent micelles after addition of sufficiant amount of lipid. We added to each protein sample appropriate amount of the lipid to reach optimal protein/lipid ratio. Unfortunately NMR measurements showed a huge decrease of signal and recording of the remaining ^{15}N signals into a narrow area. This would indicate very rigid lipid-protein micelles, which relax to fast to be recorded.

Micelle destabilisation using sonication or temperature increase led to only partial improvement, therefore we added into the sample new detergents; CHAPS and digitonin. The simple addition of CHAPS or digitonin did not destabilize micelles sufficiently and we had to remove lipids by dialysis. After dialysis signal recovered, moreover the new peaks indicated the further protein folding. A comparison of the secondary structure content with the molecular dynamics results lead to the conclusion that the combination of digitonin and octyl glucoside is the most effective combination to induce apparent protein folding.

This research was supported by AKTION Austria-Czech Republic (No. 40p2), by the Ministry of Education, Youth and Sports of the Czech Republic (MSM6007665808) and by the Academy of Sciences of the Czech Republic (Institutional research concept AVOZ60870520)

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P22

MAPPING OF THE PORE STRUCTURE OF THE VANILLOID RECEPTOR CHANNEL TRPV1

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The vanilloid receptor (TRPV1) is a nonselective cation channel that is predominantly expressed by nociceptive sensory neurons. This channel is activated by a wide array of pain-producing stimuli, including capsaicin, protons and noxious heat (> 43°C) [1]. Similar to other members of the transient receptor potential (TRP) family, the structure of the TRPV1 represents six putative transmembrane segments S1-S6, a pore region (P-loop) connecting S5 and S6, and cytoplasmic hydrophilic N- and C-terminal regions. The functional TRPV1 channel is assembled from four identical subunits. The central ion conducting pore domain is supposed to be formed by S5-S6 and the P-loop region. Structure-function studies have recently identified several key domains that contribute to the activation and the modulation of the TRPV1 receptor. However, little is known about the structural rearrangements that lead to the channel gating and ion conduction.

To obtain an insight into the pore architecture of TRPV1 and to elucidate how this region affects the ion selectivity and permeation properties of this channel, we subjected the sequence from E570 to E694 of the rat TRPV1 receptor to homology modeling. This part includes S5 and S6 helices and a loop region containing one inner helix. Based upon the predicted structure and the alignment of the pore region with related channels TRPV5, TRPV6 and KcsA [2,3], we selected three amino acids for site-directed PCR mutagenesis in order to characterize their role in channel gating and ion permeation. As the first step of the prospective cystein-scanning mutagenesis, we substituted glycine for all endogenous cysteines that are putatively exposed to the extracellular milieu. This construct was functional [4] and served as a template for the introduction of three individual cysteine substitution mutants M644C, D646C, and E648C.

HEK293T cells transiently expressing either the wild type or the respective mutant receptor were assayed by patch-clamp technique and calcium imaging. Mutation of M644C strongly reduced the magnitude of the heat induced responses, whereas the capsaicin sensitivity remained unaltered as compared with wild type. A similar pattern of responsiveness has been observed in a mutant channel in which M644 was replaced by alanine. This mutant was less permeable to Ca^{2+} exhibiting a decrease in ratio of Ca^{2+} to Na^{+} permeability from 5.0 2.4 to 1.6 0.2 (n = 4 and 10) for capsaicin activation and from 4.2 1.1 to 1.7 0.2 (n = 4 and 9) for heat stimulation. The D646C mutant appeared to be nonfunctional. Mutation of E648C rendered the channel insensitive to both thermal and chemical stimuli when applied individually; however, heat in combination with capsaicin elicited robust responses in calcium imaging experiments.

Data from this study substantiate the proposed pore organization of the TRPV1 channel and suggest that the pore-lining residues M644, D646 and E648 are important molecular determinants that govern key properties of ion permeation and channel gating.

This work was supported by Grants 305/03/0802 and 309/04/0496 of the Grant Agency of the Czech Republic, by Grant 2003CZ0020 of CSIC, Research Project of the AS CR, AVOZ 50110509 and 60870520 and by the Ministry of Education, Youth and Sports of the Czech Republic, 1M0002375201 and MSM6007665808

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P23

CHIRAL PORPHYRIN COMPLEXES AND MODELLING OF THEIR ELECTRONIC CIRCULAR DICHROISM SPECTRA

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Porphyrin core is an important component of biomolecules such as hemoglobine, myoglobine or chlorophyll. We study various porphyrin derivatives for applications in drug research, complexometry and biotechnologies. Many properties of these compounds are conveniently studied by optical (UV) spectroscopy because of the absorption properties of porphyrin chromophores. The spectra are influenced by substituents, solvent, and in some cases by bound metals. Since this variance and the relation of the spectra to molecular geometry is often poorly understood, we attempt to model spectral intensities of the electronic circular dichroism (ECD) by combined molecular mechanics/quantum mechanics (MM/QM) approach. Porphyrins have often no ECD signal because of their high symmetry; in complexes with chiral matrices, like peptides or nucleic acids, they may become chiral (optically active) and thus the spectra can reflect their interaction with the environment.

We model the geometry of porphyrins and their complexes using classical molecular mechanics models, while for spectra simulations we use semiempirical and ab initio computations. For the latter the time dependent density functional theory (TD DFT) is applied (typically at the B3LYP/6-31G* level). We plan to involve the influence of solvent by implicit and explicit solvent models. With *ab initio* parameters spectra of larger molecular complexes will be obtained by a semiempirical transition dipole coupling model (TDC).

P24

14-3-3 PROTEIN C-TERMINAL STRETCH OCCUPIES LIGAND BINDING GROOVE AND IS DISPLACED BY PHOSPHOPEPTIDE BINDING

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14-3-3 proteins are important regulators of numerous cellular signaling circuits. They bind to phosphorylated protein ligands and regulate their functions by a number of different mechanisms [1,2]. The C-terminal part of 14-3-3 protein is known to be involved in the regulation of 14-3-3 binding properties. The structure of this region is unknown; however possible location of the C-terminal stretch within the ligand binding groove of 14-3-3 protein has been suggested [3,4]. In order to fully understand the role of the C-terminal stretch in the regulation of 14-3-3 protein binding properties we investigated the physical location of the C-terminal stretch and its changes upon the ligand binding. For this purpose we have used Förster resonance energy transfer (FRET) measurements and molecular dynamics simulation. FRET measurements between Trp^{242} located at the end of the C-terminal stretch and a dansyl group attached at two different cysteine residues (Cys^{25} or Cys^{189}) indicate that in the absence of the ligand the C-terminal stretch occupies the ligand binding groove of 14-3-3 protein [5]. Our data also show that phosphopeptide binding displaces the C-terminal stretch from the ligand binding groove. Intramolecular distances calculated from FRET measurements fit well with distances obtained from molecular dynamics simulation of full length 14-3-3 protein.

This work was supported by Grants 204/03/0714 and 309/02/1479 of the Grant Agency of the Czech Republic; by Grant B5011308 of the Grant Agency of the Czech Academy of Sciences; by Research Projects 1K03020, MSM 1131 00001, and 1132 00001 of the Ministry of Education, Youth and Sports of the Czech Republic, and by Research Project AVOZ 5011922.

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P25

THIOREDOXIN SYSTEM OF STREPTOMYCES COELICOLOR: OVEREXPRESSION, ISOLATION, CRYSTALLIZATION AND CHARACTERIZATION

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Thioredoxin, thioredoxin reductase and coenzyme NADPH together form thioredoxin system, which is ubiquitous from Archaea to man. Thioredoxins (Trx) are small and very stable redox-active proteins containing a reducible disulfide bridge with amino acid sequence Cys-Gly-Pro-Cys. Trx is reduced by electrons from NADPH via thioredoxin reductase (TrxR), flavoprotein homodimeric enzyme [1]. Reduced thioredoxin acts as a major ubiquitous disulfide reductase responsible for maintaining proteins in their reduced state. Tripeptide glutathione also can act to preserve the thiol-disulfide status of proteins in the cell in a manner similar to thioredoxin. However, glutathione is not made by actinomycetes, including streptomycetes and mycobacteria, which instead contain a low-molecular-weight sugar-containing monothiol called mycothiol [2]. So, *Streptomyces* are suitable model for study of thioredoxin system.

S. coelicolor is a representative of a group of soil-dwelling, filamentous bacteria responsible for producing most natural antibiotics used in human and veterinary medicine. The 8.667.507 base pair linear chromosome of this organism contains the largest number of genes so far discovered in bacterium [3]. The complete genome sequence of *S. coelicolor* revealed several possible thioredoxin genes, and thus *Streptomyces* seems to have a more complex redox system in comparison with other bacterial species.

The presented work describes the overexpression, purification and partial characterization of thioredoxinA and thioredoxin reductase from *S. coelicolor*. The genes encoding trxA and TrxR were amplified by PCR and inserted into a pET expression vector and used to transform *Escherichia coli.* TrxA and TrxR were overexpressed by induction with IPTG as hexahistidine fusion proteins and were recovered from a cytoplasm as soluble and active proteins. Homogenous proteins were used for investigation of protein stability, their role in the redox control in the cell and study of protein-protein interaction. Thioredoxin was crystallized using the hanging drop method of vapour diffusion. We have determined the crystal structure of TrxA at 1.5 Å resolution, using a synchrotron radiation source. The protein reveals a thioredoxin-like fold with typical CXXC active site. The crystal exhibits the symmetry of space group $P2_12_12$ with unit cell dimensions 43.6 Å, 71.8Å, 33.2Å .

This work was supported by the research grants from the Slovak Grant Agency VEGA No. 1/0035/03.

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P26

PRELIMINARY RESULTS FROM CRYSTALLIZATION OF CYTOCHROMES FROM PHOTOSYNTHETIC BACTERIA *THIOCAPSA ROSEOPERSICINA*

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Cytochromes belong to the family of colored proteins that play an important role in live cells. They incorporate prosthetic group - molecule of heme - that facilitates as a member in process of electron transport. Due to this important function, it is of essential interest to study structural features of cytochromes with modern X-ray crystallographic methods.

Cytochrome *c* (cyt *c*) is a low-mass protein (26 kDa) transporting electrons among cytochrome b-c_1 complex and complex of cytochromoxidase. Cyt *c* from the purple photosynthetic bacterium *Thiocapsa roseopersicina* was isolated and purified according to Bagyinka [1]. The bacterium incorporates four different hydrogenases and three different cytochromes.

Cyt *c* was crystallized using standard crystallization methods based on vapor diffusion. Crystallization trials were performed in hanging and sitting drops [2-3] at room temperature. The most suitable concentration of protein (10mg/ml) and the precipitation agent (50% ammonium sulfate) were found. Ranging pH value higher than 7.5 the phase separation of protein was appeared. First crystal growth was observed at pH 6.0.

Particular crystallographic conditions are now being to be optimized in order to prepare monocrystals of cyt *c* suitable for X-ray diffraction measurement.

The support from AS CR (grant no. AV0260870520) and MECR (grant no. MSM6007665808) is acknowledged.

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P27

LABORATORY OF STRUCTURAL BIOLOGY AT FACULTY OF BIOLOGICAL SCIENCES UNIVERSITY OF SOUTH BOHEMIA

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The organization and research focus of the "Laboratory of Structural Biology" will be presented. The laboratory has been established in January 2004 as an inherent part of the Department of Molecular Biology at the Faculty of Biological Sciences - University of South Bohemia. Currently, the laboratory comprises of three undergraduate and three graduate students, and two independent research scientists. The research in the laboratory is divided in three areas: a) quantum chemical calculations of the NMR (nuclear magnetic resonance) spectral parameters and development of the novel schemes for interpretation of the NMR data, b) investigation of the function-structure relationships in RNA-protein complexes involved in RNA editing in *Trypanosoma brucei* – causing agent of the sleeping sickness, c) investigation of the general phylogenetic relationships among different species on the basis of the structural information. The research activities are based predominantly on: a) quantum chemical calculations (Dalton, Gaussian), b) NMR, CD, and UV spectroscopy, c) protein crystallography, and d) conventional molecular biological methods. Members of the laboratory are involved in teaching at South Bohemia University in fields of Bioinformatics and Organic chemistry. The laboratory actively collaborates with Institute of Organic Chemistry at Johannes Kepler University in Linz (Austria – prof. N. Muller), Laboratory of Pharmacological Biochemistry and Bio-organic chemistry at René Descartes University - Paris 5 (France – prof. J. Kozelka), and Department of Biochemistry and Molecular Biology Oregon Health & Science University Portland (USA- prof. M. Schumacher).

Work in laboratory of structural biology is supported by Grand agency of the Czech Republic (GACR P191; and GACR 203/05/0388) and Ministry of Education (1K04011; and LN2005 Faculty of Biological Sciences).

P28

AN EASY OPTIMALIZATION METHOD FOR *IN VITRO* PROTEIN REFOLDING – THE CASE OF CD69

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In protein biochemistry we must often solve a problem of renaturation (refolding) of proteins of our interest. Especially in the case of bacterial recombinant proteins, which are often obtained in insoluble form (the inclusion bodies), we must find an optimal way to transfer the protein molecules from denaturating agents to native conditions. We use an easy optimalization method for *in vitro* refolding of such proteins, which is critical and demanding task. Successful renaturation of the protein is a multiparameter optimalization procedure that involves finding of certain discrete conditions, such as presence of suitable additives (i.e. arginin), pH, ionic strength and redox potential of the system. These parameters are optimalized in semimicroquantitative way. Here we present results for CD69 protein [1], an early activation antigen of human lymphocytes. Efficiency of the refolding was analyzed by gel filtration and SDS-PAGE. Secondary structure of receptor CD69 prepared via such optimalized protocol was determined by drop coating deposition Raman spectroscopy method [2].

This work was supported by Ministry of Education of the Czech Republic No. MSM 0021620808, by Institutional Research Concept No. AVOZ 50200510, by Grant Agency of the Czech Republic No. 301/05/P567, and by Grant Agency of the Academy of Sciences of the Czech Republic No. A5020403.

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P29

STRUCTURAL COMPARISON OF WILD-TYPE MATRIX PROTEIN FROM M-PMV AND ITS R55F MUTANT

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Mason-Pfizer monkey virus (M-PMV) belongs to the genus of betaretroviruses which differ from lentiviruses (*e. g.* HIV-1) in a mechanism of immature capsid assembly. Matrix protein (MA) plays an essential role in different stages of retrovirus maturation. A single point mutation (R55F) in the sequence of the wt MA, however, changes the morphogenesis of the virus and capsids are assembled at the plasma membrane instead of in the cytoplasm, similarly to the lentiviruses [1].

We have focused on the study of three-dimensional structure and dynamics of both proteins (wt MA and R55F mutant) by NMR spectroscopy to reveal possible structural changes caused by the mutation (R55F). Although a structure of the wt MA has already been published as C-alpha trace [2], we decided to perform a new study of this protein to obtain a complete 3D structure. Doubly labelled (^{13}C, ^{15}N) proteins were prepared using recombinant techniques. Standard triple resonance NMR experiments were used for the assignment of resonances and three-dimensional structures of the proteins were calculated based on NMR parameters (NOE interactions, *J*- and residual dipolar couplings, etc.). Dynamical behaviour of both proteins was estimated based on ^{15}N relaxation properties. Comparison of the structure of R55F mutant with wild-type MA will be presented.

We thank the Grant Agency of the Czech Republic for the financial support (Grants 203/03/0490 and 203/04/P168).

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P30

PS II REACTION CENTER AND PHOTOSYNTHETIC PIGMENTS

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Models based on the RC PS II[1] were studied. Photosystem II reaction center (RC PS II) is a central part of the PS II complex, in which primary charge separation occurs. It consists of small number of the pigment molecules – chlorophyll *a* and pheophytin *a* molecules.

For these molecules, model calculations using ZINDO/S and TDDFT methods were utilized.

Before spectral calculations, hydrogens were added and optimized with semiempirical PM3 method. The positions of all the atoms from the referenced structure[1] of RC PS II were fixed. In the TDDFT calculations, hydrogen atoms were re-optimized using B3PW91 method with 6-31G(d) basis set.

Spectra of all the monomers of chlorophyll *a* and pheophytin *a* were estimated. Red shift was found at the ZINDO level in comparison with experimental data. However, TDDFT spectra exhibit opposite slightly blue-shifted character.

Electron transitions of selected pigment oligomers, separated from RC PS II models, were also determined. These spectra show strong multi-particle character and exhibit complex additive character.

The ZINDO determined spectra of oligomers are also in good agreement with experimental results.

In second part of our study various pigment molecules were explored. They have an important role also in other biological processes, like oxygen absorption and transport, electron transfer in photosynthesis, etc.

The calculations of electronic spectra were performed on three large groups of pigments – carotenoids, phycobilins, and chlorophylls and bacteriochlorophylls.

At first, several conformers were chosen for all the pigments. Structural database was used as an important starting point.

Optimization calculations were performed at the Hartree-Fock level with 3-21 G basis set. For the global minima, the absorption spectra were determined with the density functional theory using B3PW91 functional and the 6-31G(d) basis set further extended with diffusion functions. Their effect was found important for correct calculations of the electron transitions.

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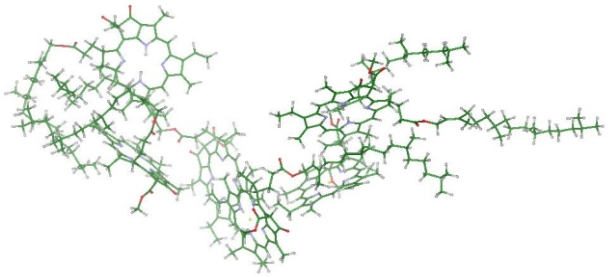

P31

THEORETICAL STUDY OF THE PLATINUM COMPLEXES INTERACTIONS WITH SULFUR-CONTAINING AMINO ACIDS

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Biologically important reactions of two cisplatin hydration products cis-$[Pt(NH_3)_2Cl(H_2O)]^+$ and cis-$[Pt(NH_3)_2(OH)(H_2O)]^+$ with sulfur-containing amino-acids cysteine and methionine were studied using density functional techniques. Considered reaction mechanism leads to formation of the chelated complexes where several monodentate intermediates were analyzed. Reaction energies were determined in the so-called supermolecule approach as well as in the non-interacting model (involving isolated molecules in reactant and product). First, all the structures were optimized using DFT method - B3LYP/6-31+G(d). For the optimized structures, single point calculations was done using slightly larger basis set (6-31++G**). Core electrons of platinum, sulfur and chlorine atoms were described by Stuttgart-Dresden pseudopotentials. Analysis of electronic density and partial charges (using NPA method) enabled a more detailed insight onto interaction energies.

In the first reaction step, the replacement of the aqua ligand was assumed where the monodentate Pt-(amino acid) was created. For the monohydrated complexes (with

chloro-ligands), it was found that in cysteine-containing systems, a formation of sulfur bonded monodentate complexes is energetically preferred to formation of Pt-N and Pt-O bonds. On the other hand, the systems with methionine slightly favors a formation of nitrogen-bonded complexes to sulfur-bonded ones. The most stable conformer from the (dihydrated) hydroxo-complexes is structure where Pt-S bond was formed. Conformers with Pt-N bonds are slightly less stable for both amino acids. Structures with Pt-O bonds are the least favorable.

The next reaction step led to the chelate formation. This process is endotermic in case of chloro species and exotermic for hydoxo compounds in supermolecular approach. The most stable chelate structures contain k(N,S) binding sites.

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ISOLATION, RECONSTITUTION AND CRYSTALLISATION OF PHOTOSYSTEM II REACTION CENTRES

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The photosystem II is a membrane pigment-protein complex consisting of over 17 subunits. It can be divided in three parts according to its function: 1. the reaction centre (RC); 2. inner and outer light harvesting complexes and, 3. the oxygen-evolving complex. RC is a minimum unit capable of charge separation between the primary electron donor (chlorophyll molecule) and primary electron acceptor (pheophytin), followed by stabilisation of separated charge by transfer of electron to plastoquinone. RC of photosystem (PS) II consists of five protein subunits (D1, D2, and subunits of cytochrome b-559 and PsbI). The heterodimer of D1 and D2 binds six chlorophylls, two pheophytins, two carotenes, two plastoquinones denoted QA and QB, respectively, and one iron atom.

The five-chlorophyll reaction centre of photosystem II (5-Chl RC PSII) was isolated from green pea (*Pisum sativum*), purified according to Vacha [1] and reconstituted with purified chlorophyll a as described in [2]. Freshly isolated and frozen samples of 5-Chl RC PSII concentrated to 15-mg/ml (1.3-mg/ml chlorophyll *a*) were subject of crystallization assays using the counter-diffusion technique implemented in single capillaries [3] and traditional sitting drops. Different types of precipitants and detergents and different pH values were tested experimentally. Optimal values (pH 7.00 and PEG4000 as a precipitant) have been already found. N-dodecyl- -D-maltoside (DM) was found as acceptable detergent. Possible green crystals were tested at synchrotron in Grenoble (beamline ID14-1) at 100K. Crystallisation experiments on PSII membrane protein complexes are still in the progress.

This work is supported by the Ministry of Education of the Czech Republic (grant MSM6007665808), by the Grant Agency of the Czech Republic (grant 206/03/D061) and by the joint C.S.I.C. and AS CR project 2004CZ0003, in the frame of the co-operation agreement 2004CZ01.

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Nový zámek v Nových Hradech

Abstracts presented in seminars Rozhovory

RTG STRUKTURNÍ ANALÝZA A PLÁNOVÁNÍ POLYMERNÍCH LÉČIV

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Metody rtg strukturní analýzy poskytují významné informace při navrhování struktur nových léčiv (drug design) [1]. Spolu s dalšími metodami, jako jsou NMR, skenovací transmisní elektronová kryomikroskopie (STEM) a počítačové modelování [2,3] se rtg strukturní analýza řadí mezi postupy SAR (Structure-activity Relationships) [4].

Jako příklad použití kombinace rtg strukturních dat, chemických experimentů a počítačového modelování je studium interakce enzymaticky degradovatelných polymerních kancerostatik s lysosomálními enzymy, kdy dochází k enzymaticky řízenému uvolňování léčiva v cílových buňkách a rozpadu hydrofilního polymerního nosiče na nízkomolekulární bloky snadno vyloučitelné z organismu.

Za modelový systém byl vybrán enzym cathepsin B a polymerní substrát obsahující poly(ethylenglykol) (PEG) a pentapeptid N^2,N^6-bis-(aspartylprolyl)lysin (DP_2K). Tento blokový polymer byl navržen jako hydrolyticky i enzymaticky degradovatelný nosič biologicky aktivních látek [5]. Kromě chemické hydrolýzy se na degradaci tohoto polymerního substrátu významnou měrou podílí i enzymem katalyzovaná hydrolýza. Rozborem rtg strukturních údajů uložených v proteinové datové bance (PDB) (www.ncbi.nlm.nih.gov) se podařilo sestavit obraz aktivního místa cathepsinu B, včetně specifických a nespecifických oblastí vazeb substrátu s enzymem, katalytické triády, okluzní smyčky a oxyanionové kapsy. Odtud bylo možno usuzovat na polohu peptidové spojky DP_2K vystavené útoku enzymu, resp. peptidové vazby podléhající hydrolytickému štěpení. Počítačový model dovoluje odhadnout i průběh Gibbsovy energie podél reakční cesty procházející postupně od nekovalentního (van der Waalsova, Michaelisova) komplexu přes transitní stav v blízkosti kovalentního komplexu dále do stavu, kdy vzniká acylenzym a rozpadá se blokový polymer. V následujícím kroku pak i té části cesty, kdy dochází k hydrolytickému rozpadu komplexu, obnovuje se počáteční stav enzymu a fragmenty polymerního nosiče jsou vyloučeny z organismu. Porovnáním výsledků chemických experimentů a výpočetních modelů je tak možno interpretovat dosažené výsledky, případně navrhovat nové systémy požadovaných vlastností.

Tato práce byla podpořena Výzkumným záměrem č. AV0Z4050913 a Klíčovým projektem č. KSK4055109.

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