3rd MEETING OF THE CZECH AND SLOVAK STRUCTURAL BIOLOGISTS

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LECTURES - MARCH 11

SOME RECENT NON-TRADITIONAL APPLICATIONS OF X-RAY SCATTERING TECHNIQUES IN BIOLOGY

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In the last decade, X-ray diffraction of protein single crystals has become quite a routine and well-known technique of their structure analysis. However, this is not the only use of X-ray scattering in biology. In this short review, several other examples will be mentioned - use of powder diffraction, X-ray reflectivity, grazing incidence, standing waves. *(see page 19 for full text)*

COMPUTER MODELING IN COMPARISON WITH X-RAY DIFFRACTION AND NMR - THREE METHODS AND THEIR RESULTS ON THE EXAMPLE OF Na/K-ATPASE

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The NMR solved structure of Na^+/K^+-ATPase N-domain [1] represents the first breakthrough in solving the structure of the ATP-binding domain with a high resolution method. However, Hilge *et al.* [1] marginalized the role of protein molecular modeling done prior to their NMR-study. Nearly three years ago we published a paper describing a computational model of the H_4-H_5 loop [2] of Na^+/K^+-ATPase. This model, and especially its improvement connected with usage of vibrational spectroscopy [3], leads to a root mean square deviation of C(RMSD) ~1.6 Å with respect to the NMR average structure [1] and 1.4 Å with respect to the recently published crystal structure at 2.6 Å resolution [4]. RMSD values of NMR structures [1] and the crystal structure [4] are all in the range of 1.4 Å and so, apart from several wrongly modeled loop regions, the computational model describes the structure correctly. Our model allowed computer docking of ATP to the N-domain and thus already at that time to identify the amino acids forming the binding pocket. Experiments based on the structural model followed and we were able to describe the binding interaction in more detail [5-7]. E446, F475, K480, Q482, K501, G502 were identified as amino acids forming the binding pocket correctly. Moreover, the aromatic stacking interaction between ATP and F475 together with one hydrogen bond of the NH2 of the adenosine moiety was described as most important for binding [6]. However, F548 was described incorrectly to be close to the binding site and one hydrogen bond was incorrectly modeled with E446 and not with Q482. Molecular modeling proposed the closest distance 3.8 Å between Q482 and ATP and thus the mechanism of participation of this residue in ATP recognition is rather indirect. Nevertheless, on basis of our experiments we could correctly state that F548 does not influence binding of ATP and that the replacement of Q482 with leucine results in a strong inhibition of both ATP binding [7]. The structurally important hydrogen bond between R423 and E472 was proposed at first on basis of our model [7]. Finally, our model had to be corrected after the publication of the NMR-structure only to a small extent and thus for several years it was the most correct structure for designing structural experiments. In spite of the fact that protein modeling is often underestimated, our work shows that molecular modeling in combination with vibrational spectroscopy and other low resolution techniques is a powerful tool for fast and relatively correct determination of protein structures in general.

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1. M. Hilge et al., *Nat. Struct. Biol.* **10** (2003) 468-474.
2. R. Ettrich et al., *J. Mol. Model.* **7** (2001) 184-192.
3. K. Hofbauerová et al., *Biopolymers* **67** (2002) 242-246.
4. K.O. Hakansson, *J. Mol. Biol.* **332** (2003) 1175-1182.
5. M. Kubala et al., *Biochem. Biophys. Res. Commun.* **297** (2002) 154-159.
6. K. Hofbauerová et al., *Biochem. Biophys. Res. Commun*. **306** (2003) 416-420.
7. M. Kubala et al., *Biochemistry* **42** (2003) 6446-6452.

DISSECTING THE ROLE OF DISULFIDE BONDS FOR FOLDING AND ENZYME-INHIBITORY ACTIVITY OF THE BOWMAN-BIRK INHIBITOR

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The Bowman-Birk inhibitor (BBI) from the soybean is a bifunctional inhibitor of trypsin and chymotrypsin/elastase characterized by a highly conserved array of 7 disulfide bonds within a relatively short polypeptide-chain of only 71 amino acids. The unusually high content of 20% of cysteines in the polypeptide chain of BBI suggests that this protein may also act as a biological storage of sulfur. The structure of BBI is characterized by a binary arrangement of an amino-terminal trypsin-reactive subdomain and a respective chymotrypsin-reactive subdomain. We have used the double-headed arrangement in the structure of BBI for assessing the functional and conformational integrity of the variants by comparative titration and activity determination experiments with trypsin. The trypsin-reactive subdomain of BBI is stabilized by 4 and the chymotrypsin-reactive subdomain by 3 S-S bonds. Interdomain disulfide linkages are not present in this molecule.

The structure of BBI displays structural peculiarities as exposed hydrophobic patches and buried ion pairs solvated by internal water molecules in the interdomain boundary. These features are in marked contrast to most other proteins comprising a hydrophobic core and exposed polar amino acids. The exposed hydrophobic patches are a structural peculiarity that is reminiscent of the kind of structural features that have been postulated to occur in partially folded proteins. We have hypothesized, that a hydrophobic collapse of the exposed hydrophobic patches into a regular hydrophobic core could be prevented by the array of seven disulfide bridges.

We have assessed the consequences of mutations in the conserved framework of S-S bonds in the trypsin-reactive subdomain for the autonomous refolding competence and the enzyme-inhibitory activity of the other subdomain directed against chymotrypsin. The deletion of disulfide bonds induces dramatic effects on the refolding competence not only of the trypsin-reactive subdomain, but also on the activity of the chymotrypsin-reactive subdomain. The variants may be distinguished into two categories. The first one displays only local irregularities as expected by hierarchical models of protein folding. In the mutants belong to the second category the chymotrypsin-inhibitory subdomain is also affected significantly in its refolding competence and its activity as a result of the mutations in the trypsin-inhibitory subdomain. Since the mutants belonging to the second category are located near ionic residues interacting across the interdomain boundary with ionic residues with opposite charge we have concluded that ionic interactions may be crucial for the kinetically controlled process of protein folding in addition to the hydrophobic effect.

AUTOMATIC BUILDING OF PROTEIN STRUCTURES

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Electron density is easily interpreted if well-phased structure factor data are available to atomic resolution. If the data are below this resolution or the phasing is poor then "bones" skeletonization and chicken wire representation of the electron density are calculated. It is necessary to use computer graphics to interpret the electron density Map interpretation is a time limiting step in the protein structure determination an is quite subjective for low-resolution maps. Its automation is an important step in the overall automation of the protein structure determination and is essential for success of structure genomic projects.

A concept of flexible fragments has been developed for automatic building of crystal structures [1]. Six monopeptides (AlphaP0, Beta1P0, Beta2P0, GammaP0, BridgeP0, CisPro0) were designed as search fragments in a phased rotation and translation function for main chain building. Electron density in a crystal and in molecular fragments is expanded in spherical harmonics and normalized Bessel functions [2, 3]. A fast rotation function, which is calculated at each grid point of the asymmetric unit, is used to find the fragment orientation. Position, orientation and internal torsion angles are refined by a phased flexible refinement. Individual fragments are connected into chains. An algorithm for chain building is simplified using generalized atoms and virtual bonds. The structure is build from flexible groups rather than from individual atoms. A sequence is aligned by a combined marker and rotamer method. The side chains are built either by a combined marker & full conformation search or by the rotamer method. Side chains are independent structure units. The protein model is built with an accuracy of about 0.2 Å at resolutions 1.2 - 2.1Å. A library of bioinorganic HET groups is currently under development. It is designed to build structures like ferredoxin and hemoglobin [4].

1. F. Pavelcik. *Acta Cryst.*, **A59** (2003) 487-494.
2. J. Friedman. *Comput. Chem.*, **23** (1999) 9-23.
3. F. Pavelcik, J. Zelinka, and Z. Otwinowski. *Acta Cryst.* **D58**, (2002) 275-283.
4. F. Pavelcik, J. Molnar. Work in progress.

PLENARY LECTURE

MAMMALIAN CLONING: FROM DOLLY TO EMBRYONIC STEM CELLS

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Since Dolly the sheep was born in 1996 the success in the production of cloned animals has been reported, after transfer of somatic cell nucleus into enucleated oocyte, in ten another mammalian species (mouse, mule, horse, rat, rabbit, cat, cow, goat, pig and interspecies-specific clone: muflon-sheep). However, the success of mammalian cloning is extremely low - approximately 3%, some of cloned animals have serious health and morphological abnormalities and this clearly speaks against the use of this procedure in humans. Nuclear transfer (cloning) does not exclusively lead to production of an embryo from which a new individual will be eventually born (reproductive cloning). More promising is so called "therapeutic cloning". Also in this case the embryo is produced. This embryo is not transferred into the uterus, but it is used for the production of so called "embryonic stem cells". It is generally believed that with these cells some presently untreatable diseases and serious injuries could be treated. However, even in this case, some problems and still unanswered questions exit.

LECTURES - MARCH 12
Morning

COMBINATION OF MOLECULAR DYNAMICS SIMULATIONS AND THERMODYNAMICS ANALYSIS: INSIGHTS INTO THE GUANINE QUADRUPLEX FORMATION AND DNA - DRUG INTERACTIONS

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Molecular dynamics (MD) simulations with explicit solvent represent very powerful tools for detailed insights into the structure and dynamics of various systems. Unfortunately, MD simulations are limited to the nanosecond timescale leading to impossibility to observe slower processes. An example of such process is the formation of a cation-stabilized guanine quadruplex (G-DNA) stem. It is an exceptionally slow process involving complex kinetics that has not yet been characterized at atomic resolution. The formation of a parallel stranded G-DNA stem consisting of four strands of d(GGGG) has been investigated using MD simulations [1]. Rather than watching for the spontaneous formation of G-DNA, our approach probed the stability of possible supramolecular intermediates (including two-, three-, and four-stranded assemblies with out-of-register basepairing between guanines) on the formation pathway. To supplement the analysis, approximate free energies of the models have been calculated using Molecular-Mechanics-Poisson Boltzmann-Surface Area (MM - PBSA) method.

Similar approach has been applied in the study of various DNA duplex sequences interacting with the minor groove binding drug 4',6-diamidino-2-phenylindole (DAPI) [2]. Sequences investigated include the binding modes observed experimentally, that is, AATT in $d(CGCGAATTCGCG)_2$ and ATTG in $d(GGCCAATTGG)_2$ and alternative shifted binding modes ATTC and AATT, respectively. The simulations also suggest that the DAPI molecule is able to adopt different conformational substates accompanied by specific hydration patterns that include long residing waters. The MM-PBSA technology has been utilized to compare free energies of different binding modes. It is demonstrated that explicit inclusion of a subset of bound water molecules shifts the calculated relative binding free energies in favor of both crystallographically observed binding modes, underlining the importance of structured hydration.

The approach applied here serves as a prototype for qualitatively investigating other molecules using molecular dynamics simulation and free-energy analysis.

1. R. Štefl, T.E. Cheatham, N. Špačková, E. Fadrná, I. Berger, J. Koča & J. Šponer, *Biophysical Journal*, **85** (2003) 1787-1804.
2. N. Špačková, T.E. Cheatham III, F. Ryjáček, F. Lankaš, L. van Meervelt, P. Hobza & J. Šponer, *Journal of the American Chemical Society*, **125** (2003) 1759-1769.

DYNAMICS OF FLEXIBLE RNA HINGES RELEVANT TO RIBOSOME FUNCTION : NON-WATSON-CRICK BASEPAIRS, MODULAR BUILDING AND SPECIFIC HYDRATION OF RNA KINK-TURNS

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Keywords: Molecular dynamics, RNA Flexibility, non-Watson-Crick basepair,modular building, long-residency hydration

Hinge-like RNA motifs occur at conserved positions in the 16S and 23S ribosomal RNAs, as revealed by x-ray crystallography of the 50S subunits of *H. marismortui* and *D. radiodurans* and the 30S subunit of *T. thermophilus*. These motifs, asymmetric internal loops, are called Kink-turns (K-turns), and are characterized by a sharp, ca. 120° bend in both phosphodiester backbones producing a V-shaped structure with an acute angle of ca. 60° between the flanking RNA helices. The bend is stabilized by non-Watson-Crick basepairs involving the minor (shallow) grooves of the helices.

We have carried out a set of explicit-solvent Molecular Dynamics (MD) simulations for selected K-turn motifs, including K-turn 38 (Kt-38) occurred in the A-site finger, Kt-42 occurred in the Factor-binding domain and Kt-58 occurred in the Domain III from the 23S ribosomal RNA of *H. marismortui*. The presence of K-turns at these key functional sites of the 50S subunit of the ribosome suggests that they confer flexibility to RNA protuberances that regulate the traversal of bound tRNAs from one binding site to another across the interface between the small and large subunit during the protein synthesis cycle. The simulations indicate that K-turns are dynamically very flexible internal loops linking geometrically rigid helical domains (modular building character of these motifs) and thus capable of regulating significant inter-segmental motions. The simulations reveal that on a nano-second timescale, K-turns sample at least two major isoenergetic conformational substates that are separated by very low energy barrier and that are stabilized by different specific long-residency hydration sites. The long-residency hydration sites stabilize non-Watson-Crick basepairs and sharp turns of the phosphodiester backbone, they mediate inter-segment contacts and may actually act as permanent waters inside these motifs. The unique flexibility of K-turn RNA motifs contrasts sharply with the rigidity of other non-Watson-Crick RNA motifs, such as the loop E (5S rRNA) and the sarcin-ricin motif (23S rRNA).

COPPER PORPHYRINS AS STRUCTURAL AND CONFORMATIONAL PROBES OF NUCLEIC ACIDS

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Complexes of water-soluble cationic (metallo)porphyrins with nucleic acids (DNA and RNA) are widely investigated owning to their possible use in photodynamic therapy of cancer. Since absorption, CD, luminescence and vibrational spectra of the (metallo)porphyrins are quite sensitive to their binding modes and local structure of the binding sites, some (metallo)porphyrins can be applied as spectroscopic probes for relaying information about local base-pair composition/sequence, structure, conformation and dynamics of nucleic acids [1]. Among various metalloporphyrins studied in complexes with nucleic acids, the copper(II) derivatives attracted a particular interest owing to their ability to form reversibly - in copper-centred (d,d) photoexcited electronic states - transient axially coordinated complexes, so-called exciplexes, with convenient electron-donating groups from their close proximity [1-5]. Due to competitive relaxation pathways asserting in non-coordinating microenvironments, and due to very fast kinetics of exciplex formation (< 1 ps), any relocation of the photoexcited porphyrin within its binding site is improbable [3]. Consequently, electron-donating groups participating as axial ligands in the exciplex formation have to be present at the proper position before the porphyrin photoexcitation. Thus, the yield of exciplex formation provides specific information about microenvironment of the porphyrin as shaped by its binding to nucleic acid in its ground electronic state. Exciplex formation of the copper porphyrins can be conveniently monitored by resonance Raman spectroscopy due to well-established exciplex Raman markers [2].

Complexes of water-soluble cationic copper(II) 5,10,15,20-*tetrakis*[4-(N-methylpyridyl)] porphyrin (CuP) with DNA-model single- and double-stranded oligo/polynucleotides consisting of various nucleobases have been studied to reveal correlation between nucleobase composition/sequence, nucleic acid structure and/or conformation, preferred binding mode and structural features of the complex, and ability to form exciplex. Our results clearly demonstrate that exocyclic C=O groups as well as endocyclic nitrogens of all nucleobases with exception of guanine residues can serve as electron-density donors [2-5], nevertheless, only if the CuP is fixed to nucleic acid otherwise then by intercalation. In such a way, CuP exciplex formation can be considered as a special marker for non-intercalative binding mode.

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1. D. R. McMillin, K. M. McNett, *Chem. Rev.* **98** (1998) 1201-1219.
2. P. Mojzes, L. Chinsky, P.-Y. Turpin, *J. Phys. Chem.* **97** (1993) 4841-4847.
3. S. G. Kruglik, P. Mojzes, Y. Mizutani, T. Kitagawa, P.-Y. Turpin, *J. Phys. Chem. B* **105** (2001) 5018-5031.
4. P. Mojzes, P. Praus, V. Baumruk, P.-Y. Turpin, P. Matousek, *Biopolymers* (*Biospectroscopy*) **67** (2002) 278-281.
5. P. Mojzes, S. G. Kruglik, V. Baumruk, P.-Y. Turpin, *J. Phys. Chem. B* **107** (2003) 7532-7535.

MD SIMULATIONS HIGHLIGHT THE ABILITY OF ANTISENSE NUCLEIC ACIDS TO HYBRIDIZE WITH THE TARGET mRNA SEQUENCE AND THEIR POTENCY TO ELICIT RNase H ACTIVITY

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In the search for efficient, more universal strategies in combating viral and malignant diseases, much effort has been invested in recent years in the development of novel approaches aimed at the suppression of gene expression. Antigene strategies pursue the gene targeting by triple-helix-forming oligodeoxynucleotides whereas the anti-mRNA conceptions comprise the use of artificial ribozymes and so-called antisense oligonucleotides complementary to the appropriate mRNA region.

The oligonucleotides with natural chemical composition have been, however, found as unsuitable for in vivo applications because of their insufficient resistance against nucleases. That is why numerous novel-type nucleotide analogs are designed, synthesized and tested [1-6]. The potential of so far reported pool of modifications of internucleotide linkages has been further enlarged by employing the nuclease-stable, isopolar, conformationally adjustable phosphonate bond, which, with its -O-P-C-O- ester ether moiety (in contrast to electroneutral methylphosphonate linkage with non-bridging P-CH_3 group), actually does not alter the original phosphodiester so much. The isopolar phosphonate analogs of oligonucleotides can be considered as an alternative to the most exploited phosphorothioate ones. They are exceptionally stable against nucleases, hybridize with the oligomer counterparts with acceptable strength, they are not chiral, and allow for a large extent of further structural tuning to provide a rich basis to modulate the conformation of the chains. Moreover, oligonucleotides consisting of both the 3-O-P-CH_2-O-5 and phosphodiester linkages are capable to elicit RNAse H activity [2].

This study is oriented on determination of the structural features altered as a result of variations in the modification of the oligonucleotide, and on their interpretation in terms of the impact of individual internucleotide linkage modification on the complexation properties of the oligonucleotide. The aim is to receive explanations at a molecular level of the biochemical/biological results. Nanosecond scale explicit solvent molecular dynamics simulations are used to verify deductions based on Raman spectroscopy results and to predict structural properties of those oligonucleotides, which would hardly be prepared in reality.

(i) The present work deals with phosphonate analogs of the natural phosphodiester internucleoside linkage (sometimes in conjunction with various aminoalkyl-linkers). Several double and triple helical complexes were used as model systems. The nucleic acids were surrounded by a periodic box of ~10000 TIP3P water atoms. Fully solvated trajectories were computed using the AMBER 5.0 software package. The implemented force field doesn't contain force constants needed to describe the modified parts of the phosphonate analogs [8]. The completion was made on the base of ab initio calculations [3].

(ii) It seemed advantageous to test the properties of synthetic compounds at the level of dinucleotides, which are readily obtainable with a wide variety of linkage modifications. Several model structures representing dodecamer triplex chains have been studied. Each model system consisted of two mutually antiparallel uridine dodecamers and a pseudostrand consisting of six modified A-dimers bound by Watson-Crick and Hoogsteen hydrogen bridges to the first and second rU12 strands (rU_{12}

(iii) Hybrid (modified:unmodified) duplexes containing a central mismatch enable to test the specificity of hybridization. Influence of the oligonucleotide modification on the stability, structural features of the mismatch site, and possible creation of "fake" base-pairing was investigated utilizing model system based on 5-d(GTG ATA TGC)-3 and 5-d(GCA TAT CAC)-3 oligomers with alternated central nucleobases and modified internucleotide linkages in their vicinity.

(iv) Triplex forming oligodeoxynucleotides have attracted a great deal of attention because of their potential use in antigene therapy. In inter molecular triplexes, third strand of ODN binds to the major groove of the DNA. However, in general, the binding of a third-strand ODN to a target DNA duplex is thermodynamically weaker than duplex formation itself. Thus much effort has been made to increase the affinity of the third strand for its target. ODN analogues carrying various aminoalkyl linkers have been synthesized, some of which have been shown to increase the thermal stability of triplexes [7]. In the present work, well known aminoalkyl linkers were tested in conjunction with various phosphonate internucleotide linkages.

(v) RNase H, an endogenous enzyme that specifically degrades target RNA in the antisense oligonucleotide/RNA hybrid duplex is an important pathway for the antisense action beside the translational arrest. RNase H hydrolyses the RNA strand in an RNA/DNA hybrid in a catalytic manner. Oligonucleotides consisting of both the 3-O-P-CH_2-O-5 and phosphodiester linkages are the only compounds [2] -

along with phosphorothioates and boranophosphates - out of hundreds of others, which are capable to elicit RNase H activity. Roots of the apparent uniqueness were interpreted by means of molecular dynamics simulations.

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1. E. Uhlmann and A. Peyman, *Chem. Rev*., **90** (1990) 544-584.
2. D. Rejman, J. Snášel, R. Liboska, Z. Točík, O. Pačes, S. Králíková, M. Rinnová, P. Kois, I. Rosenberg, *Nucleosides Nucleotides & Nucleic Acids*, **20** (2001) 819-823.
3. I. Barvík Jr., J. Štepánek, J. Bok, *J. Biomol. Struct. Dyn*., **19** (2002) 863-875.
4. I. Barvík Jr., J. Štepánek, J. Bok, *Comp. Phys. Comm.*, **147** (2002) 158-161.
5. J. Hanuš, I. Barvík Jr., J. Štepánek, P.-Y. Turpin, J. Bok, I. Rosenberg, M. Petrová, *Nucl. Acid Res.*, **29** (2001) 5182-5194.
6. I. Barvík Jr., J. Štepánek, J. Bok, *Czech. J. Phys.*, **48** (1998) 409-415.
7. N. Atsumi, Y. Ueno, M. Kanazaki, S. Shuto and A. Matsuda, *Bioorg. & Med. Chem*, **10** (2002) 2933-2939.
8. W. D. Cornell et al., *J. Am. Chem. Soc*., **117** (1995) 5179-5197.

PHOTOSYSTEM II CORE COMPLEX OF PISUM SATIVUM. CRYSTALLISATION TRIALS

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Keywords: Membrane proteins, photosystem II, *Pisum sativum*, crystallisation trials

Introduction:

Membrane proteins are responsible for enzymatic reactions, which play an important role in all fundamental processes of life. Various methods (such as electron microscopy, biochemical and biophysical spectroscopic methods, electron crystallography etc.) have been used to study proteins, yet to determine their structure, in many cases, remains problematic. The fact that protein crystals are quite unstable, highly temperature- and light -sensitive along with complicated composition of membrane proteins are responsible for difficult crystal growing and solving their structure.

Photosynthesis is a main biological energy source on which life on Earth depends. The process of photosynthesis consists of trapping of light energy by protein complexes owing to which electron transfer and proton pumping lead to a production of ATP and NADPH. Photosystem II (PS II) embedded in photosynthetic (thylakoid) membranes of cyanobacteria and chloroplasts of higher plants and algae represents one of such protein complexes. In structure, PS II is a multi-subunit complex of a molecular weight about 600kDa, containing of large number of cofactors such as molecules of chlorophylls, pheophytins, carothenoids, plastoquinons, iron and manganese which together trap, transfer and modulate a solar energy utilisation to drive reduction reactions and synthesise molecular oxygen from water [1,2].

Materials and methods:

Monomeric photosystem II core complex consisting of reaction centre proteins D1 and D2, the chlorophyll-containing inner-antenna subunits CP43 and CP47, and subunits of cytochrome b_{559}, several low molecular weight subunits, pigments (molecules of chlorophyll, molecules of pheophytin, molecule of b-carotene) and cofactors (heme and non-heme binding Fe, Mn cluster) and three extrinsic proteins of oxygen-evolving complex (manganese-stabilizing 33kDa protein, 12kDa protein and cytochrome c_{550}) [3] was isolated from *Pisum sativum*, purified and concentrated to 4-6 mg/ml chlorophyll *a* (~ 20-30 mg/ml protein). The protein solution was prepared containing additive 1mM $MnCl_2$ used in crystallisation trials [4]. Freshly isolated and frozen samples of monomeric photosystem II core complex were crystallised using the counter-diffusion technique implemented in single capillaries [5] and traditional vapour diffusion method in sitting drops. In both cases the protein solution was gelled with tetramethyl orthosilicate (TMOS) or agarose at different concentration. Gel free experiments were prepared in parallel to test the counter-diffusion technique in a clean environment and those in sitting drop were use as blanks. Commercial "MembFac^{TM}" and "Crystal Screen^{TM}" crystallisation screening kits of Hampton Research (Laguna Niguel, CA, USA) and "JBScreen Crystal Screening Kits" of JenaBioscience GmbH (Jena, Germany) as well as solutions prepared in-house were used for crystallisation trials performed at 283 K and room temperature. Different types of precipitants, inorganic salts, different pH values and variant protein:precipitant concentration ratios were tested experimentally. Optimal values of crystallisation parameters (pH around 7.00 and PEG4K as a precipitant) have been already found.

Results:

As a general observation all experiments with frozen samples of monomeric photosystem II core complex produce no crystals, only fresh purified and non-frozen protein

was suitable for crystallisation trials. Both, the use of gels and crystallisation in capillaries, were proved as useful crystallisation method.

Possible plate crystals of monomeric photosystem II core complex, were grown in sitting drops from precipitant solution containing 10% PEG4000 and 50 mM NaCl in 50 mN Bis-Tris pH 7.00 at 283 K without presence of gel. Needle-shaped crystals of protein were grown in sitting drops from the same precipitant solution in the presence of 10% TMOS and in capillaries in the presence of 0.25% agarose at room temperature. Green crystals of monomeric photosystem II core complex were measured at X13 beamline of synchrotron DESY (Hamburg) at 100K. Crystals diffracted only to 17 Å resolution.

Crystallisation experiments on PSII membrane protein complexes are still in the progress.

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1. Rhe, K. H., Morris, E. P., Barber, J. and Kühlbrandt, W.: *Nature*, 1998, **396**, 283-286.
2. Barber, J. and Kühlbrandt, W.: *Curr. Opinion in Struct. Biol*., 1999, **9**, 469-475.
3. Zouni, A., Witt, H.-T., Kern, J., Fromme, P., Krauss, N., Saenger, W. and Orth, P.: *Nature*, 2001, **409**, 739-743.
4. Adir, N.: *Acta Cryst*., 1999, **D55**, 891-894.
5. Garcia-Ruiz, J.M., Gonzales-Ramirez, L.A., Gavira, J.A. and Otalora, F.: *Acta Cryst*, 2002, **D58**, 1638-1642.

A PHENYLNORSTATINE INHIBITOR BINDING TO HIV-1 PROTEASE: GEOMETRY, PROTONATION AND SUBSITE-POCKET INTERACTIONS ANALYZED AT ATOMIC RESOLUTION

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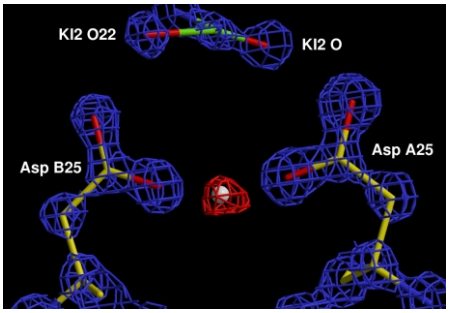
The x-ray structure of a complex of HIV-1 protease (PR) with a phenylnorstatine inhibitor Z-Pns-Phe-Glu-Glu- NH_2 has been determined at 1.03 Å, the highest resolution so far reported for any HIV PR complex. The inhibitor shows subnanomolar Ki values for both the wild?type PR and the variant representing one of the most common mutations linked to resistance development. The structure displays a unique pattern of hydrogen bonding to the two catalytic aspartate residues. The high resolution permits to assess the donor/acceptor relations of this hydrogen bonding, and to indicate a proton shared by the two catalytic residues. Structural mechanism for the unimpaired inhibition



of the protease Val82Ala mutant is also suggested, based on energy calculations and analyses.

STRUCTURE DETERMINATION OF -GALACTOSIDASE FROM ARTHROBACTER SPECIES C2-2

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The structure of cold active ß-galactosidase from Arthrobacter sp. C2-2 [1] (monomer 120 kDa having 1023 residues) was solved by diffraction techniques. Different crystallization conditions gave optically perfect crystals, however the final measurement required a specialized beam line ID29 at the source of synchrotron radiation at ESRF in Grenoble (a = 140.1, b = 205.7, c = 140.5 Å, ß = 102.3°, space group $P2_1$, 579 289 unique reflections measured to resolution 1.9 Å, Rlin = 8.5 %, completeness 95 %). Main attention is given to the special requirements for measurement of diffracted intensities, to the data processing and to structure solution procedures in the case of such a large protein.

1. Karasová-Lipovová P., Strnad H., Spiwok V., Malá Š., Králová B., Russell N. J., The cloning, purification and characterisation of a cold-active beta-galactosidase from the psychrotolerant Antarctic bacterium Arthrobacter sp C2-2, *Enzyme Microb. Tech.* (2003) 836-844.

(see page 18 for full paper)

SUBSTRATE SPECIFICITY OF HALOALKANE DEHALOGENASES

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Haloalkane dehalogenases are microbial enzymes capable to cleave carbon-halogen bond in halogenated aliphatic compounds. There is a growing interest in these enzymes because of their potential use in bioremediation, as industrial biocatalysts, or as biosensors. Structurally, haloalkane dehalogenases belong to the -hydrolase fold superfamily. Best studied haloalkane dehalogeneses are DhlA from *Xanthobacter autotrophicus* GJ10 [1], DhaA from *Rhodococcus rhodochrous* NCIMB 13064 [2] and LinB from *Sphingomonas paucimobilis* UT26 [3]. Crystal structures of these haloalkane dehalogenases are known.

There is a number of DNA sequences which are expected to code haloalkane dehalogenases. This expectation is based on sequential similiraties with known haloalkane dehalogenases: DhlA, DhaA and LinB. Protein Rv2579 from *Mycobacterium tuberculosis* H37Rv has 68 % sequence similarity with LinB. Gene rv2579 was cloned to *Escherichia col*i, protein Rv2579 was overexpressed and purified to homogeneity. Haloalkane dehalogenase activity was confirmed with pure Rv2579 enzyme.. Protein DbjA from *Bradyrhizobium japonicum* USDA110 has 41 % sequence similarity with LinB. Haloalkane dehalogenase activity of DbjA was confirmed with heterogously overexperessed pure enzyme.

Increasing number of haloalkane dehalogenase group members demands characterisation of each enzyme into more detail and substrate specificity is an important property to be evaluated Steady-state catalytic constants are being determined for the set of 31 different substrates and all currently known haloalkane dehalogenases. Multivariate statistical method - Principal component analysis [4] - will be applied on data obtained from the substrate specificity testing and the result will show structure- funcion relationships within the group of haloalkane dehalogenases. Amalgamation of structural and funcional knowledge based on in-depth studies of family of enzymes, like haloalkane dehalogenases, could bring an important and interesting knowledge into protein science.

1. Verschueren, K.H.G., Seljee, F., Rozeboom, H.J., Kalk, K.H., Dijkstra, B.W.: Crystallographic analysis of the catalytic mechanism of haloalkane dehalogenase. *Nature*, 1993. **363**: p. 693-698.
2. Newman, J., Peat, T.S., Richard, R., Kan, L., Swanson, P.E., Affholter, J.A., Holmes, I.H., Schindler, J.F., Unkefer, C.J., Terwilliger, T.C.: Haloalkane dehalogenase: structure of a Rhodococcus enzyme. *Biochemistry*, 1999. **38**: p. 16105-16114.
3. Marek, J., Vevodova, J., Kuta-Smatanova, I., Nagata, Y., Svensson, L.A., Newman, J., Takagi, M., Damborsky, J.: Crystal structure of the haloalkane dehalogenase from Sphingomonas paucimobilis UT26. *Biochemistry*, 2000. 39: p. 14082-14086.
4. Wold, S., Esbensen, K., Geladi, P.: Principal Component Analysis. Chemometrics and Intelligent Laboratory Systems, 1987. 2: p. 37-52.

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LECTURES - MARCH 12
Afternoon

A RELATIONSHIP BETWEEN SINGLE POINT MUTATION OF THE MATRIX PROTEIN FROM MASON-PFIZER MONKEY VIRUS AND ITS THREE-DIMENSIONAL STRUCTURE

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Matrix proteins (MA) play an important role in the assembly of immature viral particles in retroviruses as well as in their transportation to the cell membrane and budding. Mason-Pfizer monkey virus (M-PMV) belongs to the family of so called D-type retroviruses, which are characterized by a different mechanism of immature capsid assembly when compared with the C-type retroviruses (HIV-1). However, a replacement of arginine in the position 55 for phenylalanine in the amino acid sequence of the matrix protein results in the changed morphogenesis and the virus behaves similarly as HIV-1[1].

We started structural and dynamical studies on the R55F mutant of M-PMV MA protein to look for relations between such substantial changes of the behavior of the virus and possible changes in three-dimensional structure of the matrix protein. Doubly labeled ($^{13}C/^{15}N$) protein was expressed and purified for NMR experiments. Almost complete resonance assignment was achieved by using triple resonance NMR experiments and based on the calculation of chemical shift index four helical motifs were located. Further experimental NMR data (NOE interactions, J-couplings...) were used to calculate the three-dimensional structure of the R55F mutant. Dynamical behavior of the protein was estimated on the basis of measurements of relaxation parameters of ^{15}N nuclei.

Comparison of the solution structures of the R55F mutant with the solution structure of the wt matrix [2] revealed

a different orientation of the N-terminal half of the structural motif, which is formed by helices I and II. HIV-1 MA has been shown to form trimers [3], which might be the biologically relevant species. Molecular modeling was used to compare the propensity to form trimers of both structural motifs, i.e. wt MA and R55F, respectively.

1. S.S. Rhee, E. Hunter: *Cell*, 1990, **5**, 77-86.

2. M.R. Conte, M. Klikova, E. Hunter, T. Ruml, S. Matthews: *EMBO Journal* 1997, **16**, 5819-5826.

3. C.P. Hill, D. Worthylake, D.P. Bancroft, A.M. Christensen, W.I. Sundquist: *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 3099-3104.

STRUCTURAL MEASUREMENTS ON MEMBRANE PSBH PROTEIN

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Introduction

The psbH protein is one of key components for assembly of Photosystem II [1]. In higher plants it is one of the proteins expressed in etiolated and illuminated leaves on the same level, which indicates that it function may be considered separately from the rest of the multiprotein complex. It was thus chosen as model small protein with dominant transmembrane helix for assessment of several methods for structure determination.

Experiments

Preparation of psbH

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 ^{15}N 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 circual dichroism (CD) spectrometer.

Reconstitution and CD measurements

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.

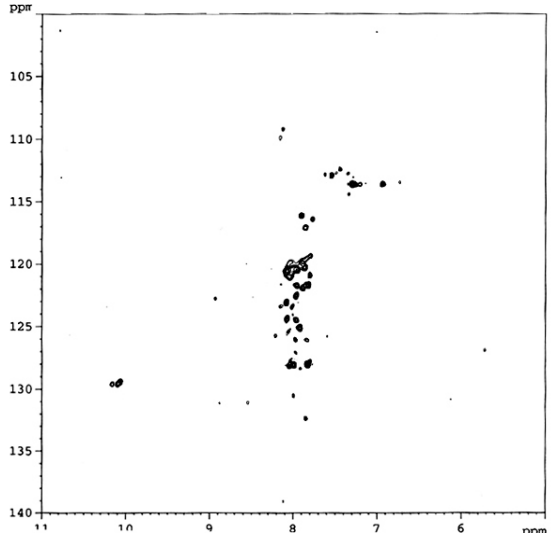

Figure 1. PsbH protein after addition of PG

NMR measurements

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 15N signals into the narrow area (figure 1). 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

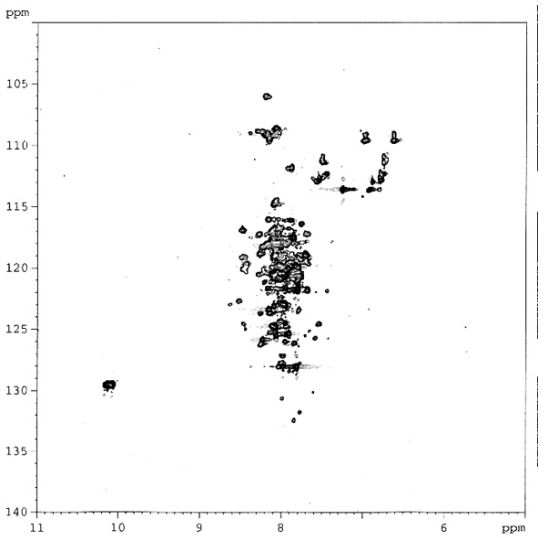

Figure 2. PsbH OG with digitonin

digitonin. The simple addition of CHAPS or digitonin did not destabilize micelles suficiently and we had to remove lipids by dialysis. After dialysis signal recovered, moreover the new peaks indicated the further protein folding. The combination of digitonin and octyl glucoside was determined as the most effective combination to induce apparent protein folding (figure 2).

Crystallisation of fusion protein and X-ray diffraction

Fusion protein GST-psbH was crystallized using standard procedures. Sufficiently large crystals were obtained. The diffraction was, however, not measurable due to problems of crystal cryoprotection. Experiments continue on improvement of crystal quality of both the fusion protein and the protein itself.

1. Komenda J., Lupínková L. & Kopecký J., *Eur. J. Biochem*., **269** (2002) 610-619.

2. Halbhuber Z., Petrmichlová Z., Alexciev K., Thulin E. & Štys D., *Protein Exp. Purif.*, **32**-1 (2003) 18-27.

DYNAMICS OF MAJOR URINARY PROTEIN I STUDIED BY NMR RELAXATION AND MOLECULAR DYNAMICS SIMULATION

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Backbone dynamics of mouse major urinary protein I (MUP-I) was studied by NMR relaxation. Data were collected for a complex of MUP-I with its natural pheromonal ligand, 2-sec-4,5-dihydrothiazole, and for the free protein. The measured relaxation rates were analyzed using the spectral density mapping. Graphical analysis of the spectral density values pro- vided an unbiased qualitative picture of the internal motions. Varying temperature greatly improved reliability of the analysis.

Quantitative parameters describing the dynamics on picosecond to nanosecond time scale were obtained using simultaneous data fitting at multiple temperatures. Both qualitative and quantitative analyses showed that the backbone flexibility on the fast time scale is slightly increased upon pheromone binding, in accordance with the previously reported results. Zero-frequency spectral density values revealed conformational changes on the microsecond to millisecond time scale. Measurements at different temperatures allowed to monitor temperature depencence of the motional parameters. Molecular dynamics simulations were performed for the free and pheromone-boun proteins using explicite solvent. Correlation functions and frequency-dependent order parameters were calculated from protein coordinates. The calculated motional parameters were compared to the results of the relaxation measurements. Combination of experimental and simulated data provided a datailed picture of the molecular motions.

This work was supported by Grant No. 203/00/0511 from Grant Agency of the Czech Republic.

RAMAN OPTICAL ACTIVITY OF SMALL PEPTIDES AND ZWITTERIONIC AMINO ACIDS

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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 solution can now be measured routinely. Because of its sensitivity to the chiral elements of biomolecular structure, ROA provides new information about solution structure and dynamics complementary to that supplied by conventional spectroscopic techniques [1].

Amino acids and peptides represent natural targets for ROA technique especially. However, the interpretation of the spectra is almost entirely dependent on *ab initio* modeling of vibrational frequencies and spectral intensities and so far imposes limits on molecular size and overall accuracy.Computation of ROA is a complex process, including evaluation of equilibrium geometry, molecular force field and polarizability tensor derivatives. Currently only a slow finite difference methods can be used for accurate evaluation of the tensors. For zwitterionic amino acids and peptides many complications arise also from their conformational flexibility and strong interaction with the solvent, which cannot be modeled with the usual procedures developed for vacuum. Many of these obstacles can be avoided in the modeling. For our ROA simulations we used simplified representation of the polarizabilities [2], Cartesian transfer techniques for the molecular tensors [3] and the COSMO continuum solvent model.

Incident circular polarization (ICP) ROA instrument has been built at Institute of Physics following the design of the instrument constructed in Glasgow [4]. However, there are currently serious limitations of the method, with respect to limited instrumental sensitivity and artifacts appearing in the spectra. Nowadays we develop program allowing to process signal in image mode and to identify and eliminate artifacts. We are going to replace conventional spectrograph in Czerny-Turner configuration with a fast stigmatic imaging spectrograph (equipped with a transmission holographic grating) [5], which enables 8 times faster acquisition of ROA signal and thus makes study of large (bio)molecular systems at lower concentration possible.

Combination of experimental and computational approaches represents unique and powerful tool for studying structure and interaction of biologically important molecules.

1. L.D. Barron, L. Hecht, E.W. Blanch, A.F. Bell, *Prog. Biophys. Mol. Bio*. **73** (2000), 1-49.

2. P. Bouř, *J. Comp. Chem.* **22** (2001), 426-435.

3. P. Bouř, J. Kapitán, V. Baumruk, *J. Phys. Chem. A* **105** (2001), 6362-6368.

4. L. Hecht, L.D. Barron, A.R.Gargaro, Z.Q.Wen, W.Hug, *J. Raman Spectrosc*. **23** (1992), 401-411.

5. L. Hecht, L.D. Barron, E.W. Blanch, A.F. Bell, L.A. Day, *J. Raman Spectrosc.* **30** (1999), 815-825.

Rho GTPases AND MOLECULAR MODELLING

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The Rho GTPases constitute a large family of small GTP binding proteins that control diverse cellular functions like the actin cytoskeleton, cell polarity, gene expression, microtubule dynamics and vesicular trafficking [1]. The understanding of their function at molecular level belongs therefore to the important tasks of molecular biology. Second reason why Rho GTPases attract the attention of scientists is that their abnormal activation plays a role in tumour invasion and metastasis [2] and in a diseases like the hypertension or bronchial asthma [3].

We will present the results of molecular modelling methods applied to some particular problems concerning Rho GTPases. First we will discuss the model of RhoA cycle at atomic level constructed on the base of known crystal structures of corresponding sub-states of the cycle. Then we will demonstrate the importance of global dynamics of proteins in their function that emerged from the functional analysis of different Rac1 isoforms [4]. Finally we will report the outcomes from the *in-silico* virtual screening focused in this case on the modifications of nucleotides that would increase their affinities towards the Cdc42 and the compounds capable to abolish the interaction of RhoA with its effector protein ROCKI and the interaction of Rac1 with its guanine nucleotide exchange factor Tiam1.

1. A. L. Bishop & A. Hall, *Biochem J.* **348** (2000) 241-255.

2. A. B. Jaffe & A. Hall, *Adv. Cancer Res.* **84** (2002), 57-80.

3. N. Wettschureck & S. Offermanns, *J. Mol. Med.* **80** (2002), 629-638.

4. L. C. Haeusler, L. Blumenstein, R. Dvorsky & M. R. Ahmadian, *FEBS Lett.* **555** (2003), 556-560.

STATISTICAL EVALUATION OF THE NEAR ATTACK CONFORMERS POPULATION

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The near attack conformation (NAC) concept was introduced by Bruice *et al*. [1]. This concept proposes that the main reason for catalytic power of enzymes is reduction of the configuration space of reacting atoms and bringing them together to a typical arrangement defined as NAC. Bruice and coworkers revealed linear relationship between log of relative rate constants (log k_{rel}) and log of NAC population concluding that the rate constants are directly dependent on the NAC population [1-3].

Haloalkane dehalogenases (HADs, EC 3.8.1.5) are bacterial enzymes catalysing the cleavage of the carbon-halogen bond in haloorganic compounds by a hydrolytic mechanism. The first step of the dehalogenation is S_N2 attack of Asp124 carboxyl group oxygen to haloalkane carbon atom followed by AdN of water molecule to ester intermediate. Bruice and coworkers postulated that NAC of the substrate molecule positioned in the active site of HAD involves two atoms forming the bond (Asp124-CO**O**$^-$…**C**-Cl). These two atoms should ideally be within the van der Waals contact distance (< 3.2 A) and the attack angle (Asp124--CO**O**$^-$…**C**-**Cl**) within ±15° deviation from the bonding angle in the TS for the S_N2 displacement of Cl^-. Here we propose effective tool for definition and calculation of NAC or any other configuration subspace population in clear statistical terms.

The NAC configuration can be described in terms of geometrical parameters, e.g. distances, angles, and dihedrals. Hence, the NAC configuration can be characterized by n-dimensional vector m made from selected *n* parameters. During the simulation the *n* selected parameters make for each time step a realization of *n*-dimensional stochastic vector n. If the system populates NAC, the realizations of vector n fluctuate with *n*-dimensional Gauss distribution with main value and covariance matrix . For each MD simulation it is possible to test a hypothesis, whether the parametrical vector is or isn't in NAC. The hypothesis can be easily tested using Mahalanobis distance representing a distance between NAC described by vector and snapshot configuration $\mathbf{n}_i$. The Mahalanobis distance has $\frac{(N \quad 1)n}{N \quad n}$ Fisher- Snedecor distribution with *n* and *N-n* degrees of freedom, where *N* is the number of steps in model simulation used for covariance matrix estimation, *n* is dimension of vectors and **n**, vector $\mathbf{n}_i$ denotes the vector **n** realization in *i*-th simulation snapshot. Finally, the accepta-

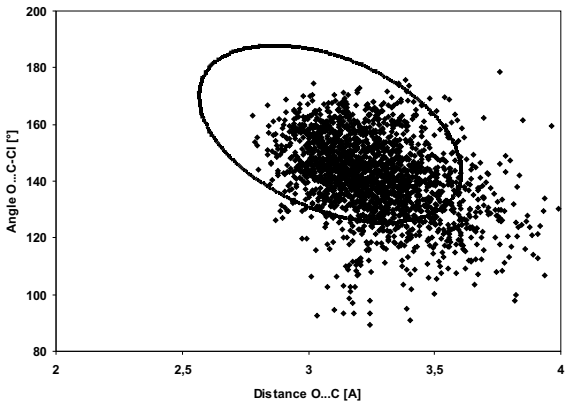

Figure. Plot of angle (Asp124--CO**O**$^-$…**C**-**Cl**) against distance (Asp124-CO**O**$^-$ … **C**-Cl) for all HAD/chlorohexane complex MD simulation snapshots. The ellipse represents critical Mahalanobis distance from NAC at which the hypothesis that the configuration does not significantly differ from NAC is acceptable (the point inside the ellipse can be considered as NAC conformation).

tion region for the hypothesis of snapshot being in NAC is represent by an unequal

$$(\mathbf{n}_i \quad)^T \quad {}^1(\mathbf{n}_i \quad) \quad \frac{(N \quad 1)n}{N \quad n} F_1 \quad (n, N \quad n).$$

Presented method is based on testing the Mahalanobis distance of a molecular arrangement from the NAC in the configuration space. The way to estimate vector as well as matrix will be discussed. Practical examples from the application of developed method on quantitative estimation of NAC for HAD will be presented.

1. F. Lightstone, T. C. Bruice, *J. Amer. Chem. Soc.*, **118** (1996) 2595-2605.
2. T. C. Bruice, F. Lightstone, *Acc. Chem. Res.* **32** (1999) 127-136.
3. T. C. Bruice, *Acc. Chem. Res.* **35** (2002) 139-148.

THEORETICAL CALCULATION OF THE NMR SPECTROSCOPY PARAMETERS ALLOW DISTINGUISH BETWEEN THE SPECIFIC DIRECT AND THE WATER-MEDIATED BINDING OF DIVALENT METAL CATION TO GUANINE.

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The calculated intermolecular and intramolecular indirect NMR spin-spin coupling constants and NMR shifts were used for the discrimination between the inner-shell and the outer-shell binding motif of hydrated divalent cations Mg^{2+} or Zn^{2+} with guanine base. The intermolecular coupling constants $^1J(X,O6)$ and $^1J(X,N7)$ ($X = Mg^{2+}$, Zn^{2+}) can be unambiguously assigned to the specific inner-shell binding motif of the hydrated cation either with oxygen O6 or with nitrogen N7 of guanine. To obtain reliable shifts in NMR parameters hydrated guanine was utilized as the reference state. The calculated change of NMR spin-spin coupling constants due to the hydration and coordination of the cation with guanine is caused mainly by the variation of Fermi-contact coupling contribution while the variation of diamagnetic spin - orbit, paramagnetic spin - orbit and spin - dipolar coupling contributions is small. The change of s - character of guanine sigma bonding, sigma anti-bonding and lone pair orbitals upon the hydration and cation coordination (calculated using the Natural Bond Orbital analysis) correlates with the variation of Fermi - contact term. The calculated NMR shifts (N7) of -15.3 and -12.2 ppm upon the coordination of Mg^{2+} and Zn^{2+} ion, are similar to the NMR shift of 19.6 ppm toward the high - field measured by Tanaka for N7 of guanine upon the coordination of Cd^{2+} cation (Tanaka, Y.; Kojima, C.; Morita, E. H.; Kasai. Y.; Yamasaki, K.; Ono, A.; Kainosho, M.; Taira, K. *J. Am. Chem. Soc.* 2002, **124**, 4595-4601). The present data indicate that measurements of NMR intermolecular coupling constants may be used to discriminate between the specific inner and outer-shell binding of divalent cations to nucleobases in DNA and RNA.

Vladimír Sychrovský, Jiří Šponer and Pavel Hobza, *J. Am. Chem. Soc*. 2004, in press.

THEORETICAL STUDY OF ^{31}P CHEMICAL SHIELDING TENSORS IN B-DNA

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National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic

Isotropic ^{31}P chemical shielding (CSI) and chemical shielding anisotropy (CSA), the quantities derived from ^{31}P chemical shielding tensors, provide valuable information on the sugar-phosphate backbone conformation in nucleic acids [1]. The backbone conformation between two successive phosphorus atoms is defined by six torsional angles. As evidenced by previous theoretical studies [2, 3, 4], the two PO ester torsional angles, (O3'-P-O5'-C5') and (C3'-O3'-P-O5') (Fig. 1), are the ones playing the prior role in determining ^{31}P chemical shielding. In addition, the effects of the OPO bond angle and CO torsional angles, e.g., (C4'-C3'-O3'-P) (Fig. 1), seem to be essential [2, 5].

In our work we apply density functional theory (DFT) to assess the influences of the , , and torsional angles, as well as the OPO bond angle on ^{31}P chemical shielding. In the geometry optimizations of a model compound (Fig. 1)

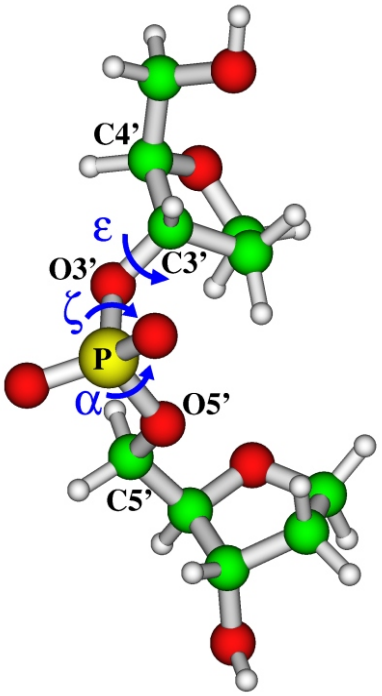

Figure 1: The model used for studying the influences of the , , and torsional angles, as well as the OPO bond angle on ^{31}P chemical shielding tensors.

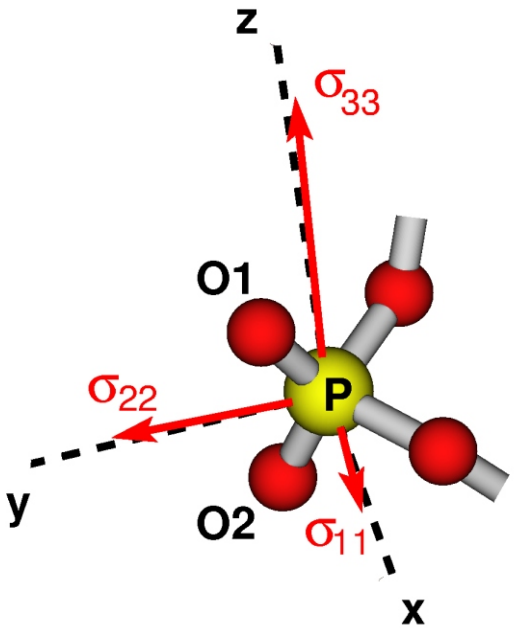

Figure 2: The orientations of the principal components of the ^{31}P chemical shielding tensor with respect to the axes of the Cartesian coordinate system.

only the torsional angle studied has been varied within its experimental range and all other backbone torsions have been kept frozen. In order to conduct NMR parameter calculations the *deMon*-NMR code [6] has been used.

Our results reveal that CSI as well as CSA increase when either or goes up from 270° to 330° for the former or from 240° to 300° for the latter. The range of CSI as a function of both torsional angles is of an order of only a few ppm. On the contrary, CSA changes within 30 ppm in the case and within 10 ppm in the case of . The larger ranges of CSI/CSA obtained for (compared with) indicate higher sensitivity of the ^{31}P chemical shielding to the a torsional angle. The plots of CSI/CSA against ( ) obtained for various values of 240°, 300° (270°, 330°) show that the trends of CSI/CSA are shifted to higher values when ( ) increases from 240° to 300° (from 270° to 330°).

Interestingly, changes in CSI introduced by the torsional angle are comparable with those introduced by and . However, leaves CSA almost unaffected. Furthermore, we have uncovered that the trend in the dependence of CSI on correlates with changes in the OPO bond angle caused by changing the torsional angle. Last but not least our calculations show that for any choice of alpha, zeta, and epsilon, the directions of the principal components of the ^{31}P chemical shielding tensors almost coincide with the axes of the Cartesian coordinate system defined so that the *y* axis bisects the O1-P-O2 angle, the *z* axis lies in the O1-P-O2 plane and is perpendicular to *y*, and the *x* axis is orthogonal to both *y* and *z* (Fig. 2).

***Acknowledgment**: This work was supported by grant LN00A016 from the Ministry of Education, Youth and Sports.*

1. D. G. Gorenstein, *Chem. Rev.*, **94** (1994) 1315-1338.
2. D. G. Gorenstein, D. Kar, Biochem. *Biophys. Res. Commun.*, **65** (1975) 1073-1080.
3. F. R. Prado, C. Giessner-Prettre, B. Pullman, J-P. Daudey, *J. Am. Chem. Soc.*, **101** (1979) 1737-1742.
4. C. Giessner-Prettre, B. Pullman, F. R. Prado, D. M. Cheng, V. Iuorno, P. O. P. Ts'o, *Bioplomers*, **23** (1984) 377-388.
5. D. G. Gorenstein, *J. Am. Chem. Soc.*, **97** (1975) 898-900.
6. V. G. Malkin, O. L. Malkina, L. A. Eriksson, D. R. Salahub: Modern Density Functional Theory: A Tool for Chemistry, J. M. Seminario, P. Politzer, Eds., Elsevier: Amsterdam, 1995.

VIBRATIONAL CIRCULAR DICHROISM OF THE NUCLEIC ACIDS

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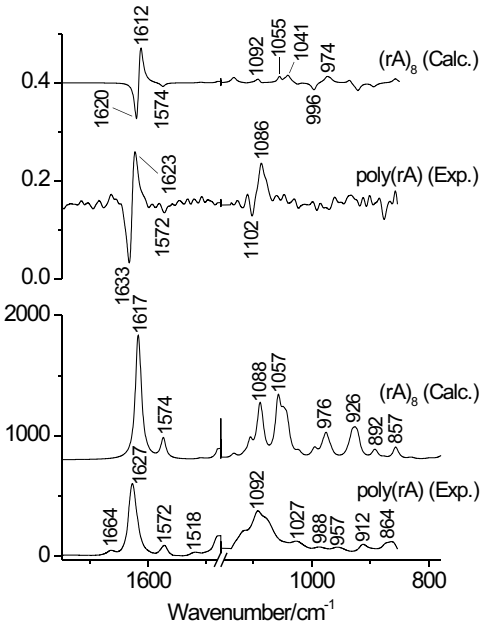
Vibrational circular dichroism (VCD) is a difference in absorption of the left- and right circularly polarized light. It is more sensitive to molecular shape than the infrared absorption spectra. For RNA and DNA structures, signs and intensities of VCD peaks depend on sequences, complexation, conformation and hydration of the molecules. Thus the technique is potentially complementary to NMR studies of solution structures, for which X-ray analysis is not possible. However, interpretation of the VCD sig-



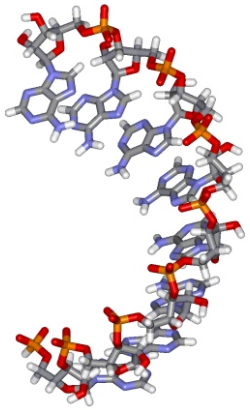


Figure: Calculated VCD (De) and absorption (e) spectra of single-stranded octamer (rA)8 and corresponding experimental VCD and absorption spectra of poly(rA).

nal requires complex computations and its accuracy is quite limited for larger molecules. We have developed a method allowing to model vibrational spectra of biopolymers [1], which was based on ab initio computations on smaller fragments, and applied previously for VCD of peptides [2]. The method enabled us to interpret spectra of B- and Z forms of DNA [3] and several RNA structures.

1. P. Bouř, J. Sopková, L. Bednárová, P. Maloň, T. A. Keiderling, *J. Comput. Chem.* **18** (1997) 646-659.
2. P. Bouř, J. Kubelka, T. A. Keiderling, T. A. *Biopolymers* **65** (2002) 45-69.
3. V. Andrushchenko, H. Wieser, P. Bouř *J. Phys. Chem. B* **106** (2002), 12623-12634.

LECTURES - MARCH 13
Morning

BACTERIAL CELL DIVISION - STRUCTURE AND FUNCTION OF PROTEINS INVOLVED

Imrich Barák* , Patrik Florek, Katarína Muchová, Ludmila Pribišová, and Zuzana Chromiková

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The basic process of cell division is conceptually similar in eukaryotic and prokaryotic cells. This process is characterized by creating the division septum between the duplicated chromosomes. There are several advantages of studying cell division in prokaryotes. In general, it is still more simple process in bacteria than in eukaryotic cells and there are several outstanding model as *Escherichia coli* and *Bacillus subtilis*. The process of cell division is intensively studied on molecular level for decades but there are still many unanswered basic questions. Probably most is known about the mechanism of cell division of rod shaped bacteria, mainly Gram-negative *Escherichia coli* and Gram-positive *B. subtilis*. Cell division, often called septation, consists of invagination of cytoplasmatic membrane and peptidoglycan synthesis. There are known a lot of players in this process but mechanism of where, how and when cells form the division septum with high fidelity is often postulated in fully different models. Probably the most controversial question in cell division of rod shaped bacteria is where to divide, in other words, how the position of the division site is determined.

The earliest event in the cell division cycle is the formation of the FtsZ ring at the future septum site. FtsZ is highly conserved GTPase with high degree of similarity with the tubulins, eukaryotic cytoskeletal proteins. During vegetative growth the FtsZ ring forms at midcell and cell divide this site. At least two distinct mechanisms are involved in accurate placement of the division machinery: the Min system and nucleoid occlusion.

Cell division as a fundamental cellular process still holds many secrets which are waiting to be unraveled. The major challenges now lie in understanding of assembly and disassembly of the protein complexes at the site of division. To understand the molecular mechanisms of these processes would require state of art experimental methods to solve the structure not only particular proteins but rather the protein complexes and their proper interpretation to explain such phenomena as asymmetry of protein localization, protein oscillation, protein spiral formation and other.

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(see page 7 for full paper)

INTERACTIONS OF Phe30 IN RUBREDOXIN DESCRIBED BY *Ab Initio* METHODS. COMPARISON WITH EMPIRICAL FORCEFIELDS

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The protein structure is to a large extent determined by the interactions of its residues. The aromatic residues are able of hybrid interactions with several partners at once. Hence there is a strong need for studying the interactions of these residues. However, the assessment of the strength of their interactions is very complicated and not that straightforward as for classical H-bond. We decided to study the interactions on the best theoretical level available on one model system. This gives us a good reference for other, "cheaper" methods and provides us with some ideas about the roles an aromatic residue can play in the protein interior.

Our primary criterion for the model system selection was a high X-ray structure quality and a presence of a densely packed cluster of aromatic residues with a phenylalanine having at least five residues closer than 4 Å. We have chosen a small (52 AAs) thermostable FeS protein rubredoxin (*desulfovibrio vulgaris*) which is assumed to serve as an electron transfer protein. The hydrophobic core of the protein is constituted of several aromatic and aliphatic residues. For the purpose of our study we have chosen Phe30 which has in its vicinity an extraordinary number of interacting partners of different character. The residue is acting at once in CH/ , , kation/ , anion/ (the sidechain) interactions as well as in a classical H-bond (the mainchain). The interaction energies of the central Phe30 with its partners represented as isolated amino acid molecules were calculated *in vacuo* using high-level *ab initio* methods. The positions of all non-hydrogen atoms were held fix at the X-ray structure geometry, while the position of all hydrogen atoms was optimized at the DFT/6-31G** level. The interaction energy was then calculated as the sum of two contributions; one being an RI-MP2 interaction energy extrapolated to the basis set limit, the other a correction term for higher order (CCSD(T)) correlation energy. As a test of the reliability of various empirical potentials we have computed the interaction energies of the same systems in several common forcefields.

Our present results show that there is a strong interaction of Phe30 with the surrounding residues without any repulsion. Such strength could be the reason for thermostability of the protein and also an origin of the folding process. There is pronounced difference in the performance of the tested forcefields. Some forcefields proved to be able to reproduce the results of the reference method.

THE IMPORTANCE OF HAVING GLYCOSYLATED CLEAVABLE PROPEPTIDE: THE STORY OF ß-N-ACETYLHEXOSAMINIDASE

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Fungal ß-*N*-acetylhexosaminidases (chitobiases, EC 3.2.1.30) are secreted enzymes that hydrolyze chitobiose into monosaccharides. The enzymes are physiologically important for the formation of septa in molds, germ tubes in yeast, and fruit bodies in fungi [1]. Moreover, they have found many applications in biotechnologies due to their unique abilities to synthesize new and unusual oligosaccharide structures [2].

We have cloned and sequenced ß-*N*- acetylhexosaminidase from the collection strain of *Aspergillus oryzae* CCF1066 [3]. This enzyme is composed of four polypeptide segments. The *N*-terminal signal peptide is cleaved intracellularly. The zincin-like and catalytical domain define the enzyme as a member of family 20 glycohydrolases. Homology modeling revealed significant similarity with the two crystallized bacterial ß-*N*-acetylhexosaminidases.

Fungal ß-N-acetylhexosaminidases contain a unique N-terminal propeptide that is processed intracellularly before the secretion of the enzyme. Detailed pulse-chase and inhibition studies revealed that the propeptide is processed very early during the biosynthesis, just after the addition of N-glycans. The propeptide must be processed in order to asssist in enzyme refolding, activation, and dimerization. Monomeric enzyme subunits devoid of the propeptide are inactive, cannot dimerize, and may not be secreted from the cell [4]. Dimers containing a single propeptide are secreted at only half the rate of those containing both propeptides, and have lower specific activity [5].

The unique propeptide properties are undoubtedly dictated by its unusual structural features. Therefore we turned our attention toward the characterization of its primary structure. The propeptide has a prolin rich C-terminal part with several potential O-glycosylation sites. The glycosylation pattern is extremely difficult to solve as there are at least two or three glycans attached to serin or threonin. Moreover, the glycosyaltion appeared rather resistant to chemical cleavage and it also protected the C-terminal part of the propeptide from proteolysis. Partial primary structure including glycosylation was solved by tandem mass spectrometry, namely collision induced dissociation (CID). Final characterization using electron capture dissociation (ECD) combined with high resolution and high mass accuracy experiments on an FT-ICR mass spectrometer is under progress. [6]

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1. V. M. Hearn, G. M. Escott, E. Glyn, V. Evans, D. J.Adams, *Microbios*, **93** (1998) 85-104.
2. L. Weignerová, P. Vavrušková, A. Pišvejcová, J. Thiem, V. Křen, *Carbohydr.Res*., **338** (2003) 1003-1008.
3. L. Hušáková, E. Herkommerová-Rajnochová, T.Semeňuk, M. Kuzma, J. Rauvolfová, V. Přikrylová, R. Ettrich, O. Plíhal, K. Bezouška, V. Křen, *Adv.Synth.Catal*., **345** (2003) 735-742.
4. O. Plíhal et al., manuscript in preparation.
5. J. Sklenář et al., manuscript in preparation.
6. P. Man et al., manuscript in preparation.

CLASSES OF RNA CONFORMATIONS

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The diversity and complexity of RNA structure is a consequence of the high flexibility of the polynucleotide backbone and is best exemplified by the crystal structures of the ribosomal units. A nucleotide has seven torsional degrees of freedom, including the torsion c around the glycosidic bond; this multidimensionality of the nucleotide conformational space represents a major obstacle to its systematic analysis. In the work presented here, the multidimensional RNA conformational space and the very large number of possible correlations among the individual torsion angles were simplified by focusing on the interrelationships of the conformation angles that define the phosphodiester linkage (torsion angles labeled $_{i}$ and $_{i+1}$) and the other backbone torsion angles. A single near atomic resolution structure with over 2800 nucleotides from the 23S and 5S rRNA molecules of the large ribosome subunit (NDB code RR0033, PDB code 1JJ2, ref. 1) serves as a database for the analysis.

Detailed analysis of the RNA backbone torsions was performed in six three-dimensional projections of the torsional multidimensional space. Each 3D torsional map consists of a distribution of points (t1, t2, t3)i that are Fourier-transformed (FT) into their pseudo electron densities, density maps visually inspected to localize peak positions and peak maxima fitted. Before the FT averaging, the original data matrix of 2841 points had to be modified. A majority (~70%) of all nucleotides of RR0033 are in the A-type conformation with torsion angle values at the phosphodiester link $_{i}$ ~ 290° and $_{i+1}$ ~300°. These residues were excluded from the original data matrix because concentration of most points into a narrow area of the map would deform the pseudo electron densities in other regions. The remaining 830 points were Fourier-averaged and further analyzed.

In each of the six analyzed maps, about ten peak maxima were identified, their positions fitted and named. Distances between the peaks and 830 individual data points ($_{1}$, $_{2}$, $_{3}$)i of each map allow labeling of data points by the name of the nearest peak. Data points labeled by peak names in the six maps were clustered by a technique called "lexicographical clustering" which starts by alphabetical sorting of the data point labels for the six maps in the same way as one would order words in a dictionary. To make sure lexicographical clusters represent conformational families, clustered dinucleotide fragments were compared by the standard least square overlap of dinucleotide atoms and outliers removed; rmsd values of the families were 0.2 - 0.7 Å. Most dinucleotides in the families are conformationally so similar that all their torsions could be and were determined and their averaged Cartesian coordinates determined. The identified conformations will be characterized in the talk and the accompanying poster. Here we summarize a few most interesting findings.

Non-A-type conformations occur in most cases isolated between nucleotides in A-type conformations and rarely connect to one another. Especially several "open" conformations (numbers 8-17) occur in single stranded regions linking two or more double helices, Some other conformations with stacked bases (as #1, 3-6) can be a part of double helical regions with local disruption of the helix by a bulge or non-canonical base pair(s).

Sequence preferences were observed only in a few conformational families. Notably, the recognized preferences involve preference for purine rich regions in conformations #4-6 (preference for RR) and #2, which occurs preferentially in tetra loops with sequences RNRN. In contrast, the conformation with parallel orientation of the subsequent bases and zero rise known as "adenine platform" motif (#7) showed no sequence preference for AA.

Stacked or parallel bases, 'normal' rise.

Conformation #1: backbone conformation is, in fact, very close to that of the purine-pyrimidine (RY) steps of Z-DNA but in contrast to the Z-DNA, both bases have 'normal' *anti* orientation and the conformation shows no sequence preference for RY known from the Z-DNA. Conformation #2: an unusual combination of torsions $_{i}$ - $_{i+1}$ - $_{i+1}$ - $_{i+1}$ reverses the direction of the backbone at the beginning of the second nucleotide so that the second ribose is flipped upside down from its A-type position, the second base is rotated anticlockwise from its A-type position by ~180°, and the bases do not stack. The conformation has a preference for short, mostly tetra-, loops with prevailing sequence RNRN. It is most often located at the stem-loop interface and one nucleotide of the motif forms a non-canonical pair, typically GoA, of a tetra loop.

Parallel bases and low-to-zero rise.

Conformations #5 - 7: bases have low rise, are in edge-to-edge orientation and can form non-canonical hydrogen bonds directly or *via* a water molecule. A significant feature of #5 - 6 is that their dinucleotides can occur at

the opposite strands of double helices with non canonical base pairs and prefer purine rich regions, the motif itself has mostly RR sequence. They can also occur in single stranded links. The family #7 is very similar to the motif known as the "adenine platform" but it shows no sequence preference for AA.

"Open conformations": not stacked bases, short-to-normal P_i-P_{i+2} and large $C1_i$-$C1_{i+1}$ distances.

Conformations #8 - 9: the backbone forms a U-shaped turn in the RNA direction with short P_i - P_{i+2} distances. The second base is rotated 180° away from its position in the A-type but lies in the same plane, conformation #9 has the first base in the minor syn orientation. Dinucleotides of families 8 - 9 participate mostly in single stranded links or bulges between double helical regions and form base pairs only rarely, never are involved in the canonical ones.

Conformations #14 - 17: are extremely extended, the bases are rotated away from each other, the first base is 'above' the P_i and the second 'below' the P_{i+2}, and border the dinucleotide on both ends. Positions of the base and phosphate attached to the second ribose are swapped and the backbone of the dinucleotides has an S shape form. Similarly to other "open" conformations, #14 - 17 form hinges between a short single stranded link and a double helix.

Conformations #19 - 32: Nucleotides in the A-type conformations form about 70% of the studied ribosomal RNA and were further investigated. Their large majority, exactly 1513, have the whole dinucleotide in conformation of the canonical A-RNA. There are, however, about fifteen other well defined conformational families with small but pronounced deviations from the canonical A-RNA. These deviations are localized in one or two torsion angles of the first or second nucleotide.

The present work suggests that the *multidimensionality of the RNA* conformational space can be approached by analysis of conformations at the phosphodiester link $O3'_i$ - P_{i+1} - $O5'_{i+1}$, defined by torsion angles $_i$ - $_{i+1}$. We deduced the central role of torsions $_i$ - $_{i+1}$ from the fact that they exhibit the highest variability yet are limited into well defined regions, noise notwithstanding. We suggest that character and importance of the $_i$ - $_{i+1}$ scatter gram can be compared to the cornerstone of protein structural science, Ramachandran plot of the protein backbone torsion angles and .

1. Ban, N., Nissen, P., Hansen, J., Moore, P.B. and Steitz, T.A. (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science*, **289**, 905-920.

IS EF-TU AS A WELL-UNDERSTOOD BACTERIAL MOLECULAR SWITCH STILL A PUZZLE FOR STRUCTURAL BIOLOGISTS?

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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. Besides this, EF-Tu is proposed to function in other parts of the cell metabolism as well. 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 cytoplasmic protein, however, thanks to some of its below listed features, it may be considered as a special case.

There have been described several post-translation modifications of the protein. 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. EF-Tu was also described recently as a major component of cytoskeleton like structures in *Mycoplasma pneumoniae* cells and most importantly it was identified there as a protein anchored in the membrane and binding fibronectin which is a multifunctional protein interacting with molecular motor like structures in eukaryotes.

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 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 us-

ing above mentioned method and used them in comparative studies in order to better understand 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.

For the future we will compare sequences of Tuf-1, gene coding active EF-Tu, from both strains mentioned above and we will try to find why EF-Tu from *S. aureofaciens* is capable of spontaneous aggregation.

POSTERS

MOLECULAR DYNAMICS STUDY OF ENZYME DHAA REACTIVITY WITH 1,2,3-TRICHLOROPROPANE

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Biological degradation of 1,2,3-trichloropropane (TCP) is studied in the context of removal of halogenated hydrocarbon from the environment as well as from the reaction mixture formed during epichlorohydrine production. The bacterial enzyme haloalkane dehalogenase DhaA from *Rhodococcus* sp. (EC 3.8.1.5) transforms TCP to 2,3-dichloropropane-1-ol. This reaction step is the slowest in TCP mineralization. One possibility leading to increased activity of DhaA is modification of the enzyme by mutations. Directed evolution method resulted in two mutations (C176Y and Y273F) increasing the enzymatic activity [1].

The objective of this work was to explain increased enzymatic activity of DhaA mutants by molecular dynamics (MD) simulations. Molecular docking method suggested three different TCP binding modes within the DhaA active site (bm1, bm2 and bm3). Therefore, nine MD simulations of DhaA/TCP complex were run: three simulations (bm1-bm3) per each enzyme (wild type - WT-DhaA; mutant C176Y - M1-DhaA; and mutant C176Y+Y273F - M2-DhaA). The reactive center on the substrate molecule was specified as well as corresponding product for all binding modes. The bm1 is expected to lead to 1,3-dichloropropane-2-ol, bm2 to (*S*)-2,3- dichloropropane-1-ol and bm3 to (*R*)-2,3-dichloropropane-1-ol.

The TCP reorientation from bm1 to bm2 during the first 500 ps was observed in all simulation starting from bm1. Therefore, it can be concluded that bm1 is a metastable state. This observation is in a good agreement with experimental data. Furthermore, hydrogen bond interaction was observed between Tyr273 hydroxyl and Asn41 backbone carbonyl in the WT-DhaA and M1-DhaA simulations. This interaction is not possible in M2-DhaA mutant allowing Asn41 backbone carbonyl hydrogen bond interaction with the catalytic water molecule. It causes better orientation of the catalytic water for the second reaction step (AdN addition of the catalytic water to intermediate ester group). The change in the catalytic water orientation is probably the main effect of the Y273F mutation on increased DhaA activity. The C176Y mutation causes the increase in the attraction between the residue 176 and Phe144 and decrease in the attraction between residue 176 and Lys175. The upper tunnel is one of the entrances to DhaA active site and the C176Y mutation can influence substrate, products, and/or water exchange between bulk and active site.

1. T. Bosma, J. Damborsky, G. Stucki, D.B. Janssen: *Appl. Environ Microbiol*, **68** (2002) 3582-3587.

A MOLECULAR DYNAMICS STUDY OF THE CYCLIN-DEPENDENT KINASE-2 (CDK2) WITH SUBSTRATE PEPTIDE (HHASPRK), INHIBITION OF CDK2 BY PHOSPHORYLATION

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The cyclin-dependent kinase, CDK2, regulates the eukaryotic cell cycle at the G1 - S boundary. CDKs activity is regulated by complex mechanism including binding to positive regulatory subunit and phosphorylation at positive and/or negative regulatory sites [1]. For activation CDK2 requires binding to Cyclin A or Cyclin E. The CDK2 obtains full activity after phosphorylation of the threonine residue (T160) in the activation segment (T-loop) [2]. CDK2 catalyzes the phosphoryl transfer of the adenosine-5′-triphosphate (ATP) g-phosphate to serine or threonine hydroxyl in the protein substrate. The CDKs activity is inhibited in several ways, for example, by (de)phosphorylation, interaction with various natural protein inhibitors [3, 4], etc. The CDK2 can be negatively regulated by phosphorylation at Y15 and, to a lesser extent, at T14 in the glycine-rich loop (G-loop) [5].

This work describes behavior of the fully active CDK2 (pT160-CDK2/Cyclin A/ATP complex) with substrate peptide (HHASPRK) and CDK2 inhibited by phosphorylation at T14, Y15, and T14/Y15 residues altogether in the G-loop using molecular dynamics simulations with the Cornell et al. force field as implemented in the AMBER software package [6]. Inhibited complexes of CDK2 were prepared from pT160-CDK2/Cyclin A/HHASPRK/ATP (1QMZ PDB ID code) by phosphorylation of the T14 and/or Y15 residues. Enzyme dynamics was studied during

8 ns long trajectory. Differences in conformational behavior of key residues for substrate binding and phosphoryl transfer of fully active vs. inhibited CDK2 will be presented.

1. D. O. Morgan, *Annu. Rev. Cell Dev. Biol.*, **13** (1997) 261-291.

2. D. P. Jeffrey, A. A. Russo, K. Polyak, *Nature*, **376** (1995) 313-320.

3. M. Otyepka, Z. Kříž, J. Koča, *J. Biol. Struct. Dyn*., **2**0 (2002) 141-154.

4. T. D. Davies, D. J. Pratt, J. A. Endicott et al., *Pharmacol. Ther*., **93** (2002) 125-133.

5. H. L. De Bondt, J. Rosenblatt, J. Jancarik, *Nature*, **363** (1993) 595-602.

6. D. A. Case et al., AMBER ver. 6.0, University of California, San Francisco, 1999.

THE ROLE OF HALIDE-STABILIZING RESIDUES IN HALOALKANE DEHALOGENASES STUDIED BY QUANTUM MECHANIC CALCULATIONS AND SITE-DIRECTED MUTAGENESIS

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Haloalkane dehalogenases are bacterial enzymes catalyzing the hydrolytic cleavage of the carbon-halogen bond of various halogenated aliphatic hydrocarbons present in the environment as dangerous pollutants. This catalytic process results in the formation of an alcohol, halide and proton as the reaction products. Catalytic triad, oxyanion hole and halide-stabilizing residues were previously found to be the three main structural features essential for the catalytic performance in these enzymes [1]. Halide-stabilizing residues are not structurally conserved among different haloalkane dehalogenases. The difference in halide-stabilizing residues between two studied haloalkane dehalogenases with known structure was tested in present study using quantum mechanical calculations and site-directed mutagenesis.

Nucleophilic substitution (SN2) as the first reaction step of dehalogenation process was modeled and the level of electrostatic stabilization of the transition state structure and released halide ion provided by each of the active site residues was calculated. Studied systems were the enzymes DhlA from the soil bacterium *Xanthobacter autotrophicus* GJ10 and LinB from *Sphingomonas paucimobilis* UT26 and 1-chlorobutane substrate docked into the active site of the protein. Our *in-house* program TRITON [2, 3] interfacing semi-empirical quantum mechanical package MOPAC [4] and homology modeling package MODELLER [5] was used for all calculations. Presented results showed that some of the studied residues can be assigned as primary (essential) and some as secondary (less important) halide-stabilizing residues. Consecutively both theoretical and experimental site-directed mutagenesis was conducted with LinB enzyme to confirm location of its primary and secondary halide-stabilizing residues. Asn38Asp, Asn38 Glu, Asn38Phe, Asn38Gln, Trp109Leu, Phe151 Leu, Phe151Trp, Phe151Tyr and Phe169Leu mutants of LinB were theoretically modeled and simultaneously experimentally constructed, purified and kinetically characterized.

Based on the results the following active site residues were classified as the primary halide-stabilizing residues: Trp125 and Trp175 of DhlA, and Asn38 and Trp109 of LinB. All these residues stabilize the halide ion released from the substrate molecule by a hydrogen bond and their substitution significantly modified catalytic properties of mutated enzymes. Phe172, Pro223 and Val226 of DhlA and Trp207, Pro208 and Ile211 of LinB residues were classified as the secondary halide-stabilizing residues with no significant effect on the catalysis. The good qualitative agreement between modeled stabilization effect and catalytic activity for studied mutants of LinB was observed [6] confirming the applicability of TRITON for similar type of studies. Program TRITON is currently available for Irix, Linux and NetBSD operating systems and is provided free of charge for academic users. For more information and program download see the web page
http://ncbr.chemi.muni.cz/triton/triton.html.

1. Verschueren, K. H. G., Seljee, F., Rozeboom, H. J., Kalk, K. H., and Dijkstra, B. W., *Nature* **363** (1993) 693-698.

2. Damborský, J., Prokop, M., and Koča, J., *Trends in Biochemical Sciences* **26** (2001) 71-73.

3. Prokop, M., Damborský, J., and Koča, J., *Bioinformatics* **16** (2000) 845-846.

4. Stewart, J. J. P. *Journal of Computer-Aided Molecular Design* **4**, (1990) 1-45.

5. Sali, A., *Molecular Medicine Today* **1** (1995) 270-277.

6. Boháč, M., Nagata, Y., Prokop, Z., Prokop, M., Monincová, M., Koča, J., Tsuda, M., and Damborský, J., *Biochemistry* **41** (2002) 14272-14280.

STRUCTURE OF THE -*N*-ACETYLHEXOSAMINIDASE 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* and *S. plicatus* (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 docking of the chitobiose was explored with the DOCK module of SYBYL. 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. To test thermostability of the enzyme, Raman spectra in the 5-60 °C temperature range were recorded and analyzed by means of factor analysis.

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1. Q. Cheng, H. Li, K. Merdek, J. T. Park, *J. Bacteriol*., **182** (2000), 4836-4840.
2. A. Sali and J.P. Overington, *Protein Sci.*, *3* (1994), 1582-1596.

GUANINE QUADRUPLEX LOOPS STUDIED BY MOLECULAR MECHANICS AND DYNAMICS TOOLS: ADVANCES AND FORCE FIELD LIMITATIONS

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Wide range of methods has been applied to various G-DNA sequences that are able to form four-stranded arrangement. Computational methods contribute to the experimental ones and help them in prediction and explanation of structural features which can influence quadruplex formation and stability.

A computational analysis of d(GGGGTTTTGGGG)2 guanine quadruplexes containing both lateral and diagonal four-thymidine loops was carried out using Molecular Dynamics (MD) simulations, Locally Enhanced Sampling (LES) simulations, systematic conformational search, and free energy Molecular-Mechanics-Poisson Boltzmann-Surface Area (MM-PBSA) calculations with explicit inclusion of structural monovalent cations. The study provides a qualitatively complete analysis of the available loop conformational space. Major conformational transitions not seen in conventional MD simulations are observed when LES is applied. The favored LES structures consistently provide lower free energies (as estimated by MM-PBSA) than other structures. Unfortunately, the predicted optimal structure for the diagonal loop arrangement differs substantially from the X-ray experiments. This result is attributed to force field deficiencies, such as the potential misbalance between solute - cation and solvent - cation terms. The optimal diagonal and lateral loop arrangements appear to be close in energy though a proper inclusion of the loop monovalent cations could stabilize the diagonal architecture.

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MOLECULAR MODELLING STUDY OF INTERACTION OF CYS2 DOMAIN OF PROTEIN KINASE C WITH ITS ACTIVATOR PHORBOL 12-MYRISTATE-13- ACETATE AND LIPID BILAYER

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Protein kinase C (PKC), a family of serine/threonine kinases, is intimately involved in the regulation of a variety of cellular functions such as gene expression, cellular growth and differentiation. PKC is regulated by two distinct mechanisms: by binding 3 molecules of ATP which regulates the active site and subcellular localization of the enzyme, and by second messengers (Ca^{2+}, diacylglycerol, phorbol esters) which promote PKC's membrane association and result to pseudosubstrate exposure. Phorbol 12-myristate-13-acetate (PMA), powerful tumor-promoting agent, binds to the Cys1 and Cys2 domain in PKC on membrane surface with nanomolar binding affinity. Molecular modelling [1] was used for the study of PMA+dipalmitoylphosphatidylcholine (DPPC) bilayer and Cys2+PMA+DPPC bilayer complexes. Function of hydrophobic and hydrogen bond interactions between PMA and Cys2 domainin is discussed. Comparison with existing experimental data showed that obtained molecular model of Cys2 domain with its activator PMA on membrane surface is consistent with existing X-ray [2] and NMR [3] results.

1. E. Lindahl, B. Hess & D. Van der Spoel, *J. Mol. Mod.*, **7** (2001) 306-317.
2. G. Zhang, M. G. Kazanietz, P. M. Blumberg & J. H. Hurley, *Cell*, **81**, (1995) 917-924.
3. R. X. Xu, T. Pawelczyk, T.-H. Xia & S. C. Brown, *Biochemistry*, **36** (1997) 10709-10717.

RATIONAL REDESIGN OF ENZYMES USING PROGRAM TRITON

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Engineering of enzymes to improve their catalytic properties is one of the present-day challenges of biochemistry and molecular biology on the side of experimentalists and molecular modeling on the side of theoreticians. The rational engineering of a given enzyme requires an understanding of the structural features determining its catalytic efficiency. In particular, a protein engineer has to know which amino acid residues of the protein are involved in the catalysis and how to modify them to achieve an increased activity. The three-dimensional structure of the protein or protein-substrate complex is usually required for protein engineering using molecular modeling.

Our laboratory has been developing program TRITON as a graphical tool for modeling protein mutants and assessment of their activities [1, 2]. TRITON serves as the simple and effective graphical interface for preparation of input data for calculation and analysis of outputs. Mutants are constructed from the wild type structure as the template by homology modeling using program MODELLER [3]. Then the process of enzymatic reaction taking place both in the wild type and in the mutant active site is modeled using the semi-empirical quantum mechanical program MOPAC [4]. Semi-quantitative predictions of mutant activities are achieved by evaluating the changes in the activation energies of the system associated with the mutation, changes of partial atomic charges on selected atoms and electrostatic interaction energies of the active site residues with the substrate during the reaction. The applicability of TRITON has been extensively tested using bacterial enzymes haloalkane dehalogenases as model systems [5-9].

TRITON has primarily been developed for molecular biologists and biochemists, non-specialists in computer modeling. Preparation of calculations using the wizards implemented in the TRITON is very easy even for beginners in computer modeling. The program TRITON can be run under operating systems IRIX, Linux and NetBSD and is provided free of charge to the academic users. For more information about TRITON see the web page http://ncbr.chemi.muni.cz/triton/triton.html.

1. Damborský, J., Prokop, M., and Koča, J., *Trends in Biochemical Sciences* **26** (2001) 71-73.
2. Prokop, M., Damborský, J., and Koča, J., *Bioinformatics* **16** (2000) 845-846.
3. Sali, A., *Molecular Medicine Today*, **1** (1995) 270-277.
4. Stewart, J. J. P., *Journal of Computer-Aided Molecular Design*, **4** (1990) 1-45.
5. Damborsky, J., Kutý, M., Němec, M., and Koča, J., *Journal of Chemical Information and Computer Sciences*, **37** (1997) 562-568.
6. Kutý, M., Damborský, J., Prokop, M., and Koča, J., *Journal of Chemical Information and Computer Sciences,* **38** (1998) 736-741.
7. Damborsky, J., Boháč, M., Prokop, M., Kutý, M., and Koča, J., *Protein Engineering,* **11** (1998) 901-907.
8. Boháč, M., Nagata, Y., Prokop, Z., Prokop, M., Moninco-vá, M., Koča, J., Tsuda, M., and Damborský, J., *Biochemistry* **41** (2002) 14272-14280.
9. Kmuníček, J., Boháč, M., Luengo, S., Gago, F., Wade, R. C., and Damborský, J., *J Computer-Aided Molecular Design* **17** (2003) 299-311.

ACCURACY AND INFORMATIONAL CONTENT OF SPECTROSCOPIC METHODS FOR DETERMINATION OF THE SECONDARY STRUCTURE OF PROTEINS EXPRESSED AS A PART OF THE GENE FUSION SYSTEM

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The main aim of this study is to characterize accuracy of the standard spectroscopic methods, i.e. circular dichroism (CD), Fourier transform infrared (FTIR) and Raman spectroscopy, for determination of the secondary structure of the proteins, which are co-expressed with a soluble protein. In many cases when proteins are expressed in gene fusion systems it seems useful to keep a soluble protein covalently bound to the studied protein. The reason is that an acquisition of the desired protein represents thrombin cleavage from the fusion protein, which cause smaller yield of the purified protein. Moreover, the cleavage site could exist in the expressed protein or the protein alone is insoluble.

We examined the glutathione S-transferase (GST) gene fusion system, which belongs to the most widely used systems for expression, purification and detection of proteins in *E. coli*. The system is based on intracellular expression of genes or gene fragments co-expressed with *S. japonicum* GST [1]. The model protein that was co-expressed with GST in our study was N-domain of H_4-H_5 loop of Na^+/K^+-ATPase. The mass ratio of the N-domain to GST protein was approximately 1:1. Prepared samples were N-domain alone, GST alone and GST covalently bond to N-domain. It gives a chance to compare results from spectroscopic measurements of the independent proteins, which should be the sum for the GST covalently bond to N-domain.

CD spectra were analysed for the secondary structure content by means of the Dichroweb website [2]. All published analyses of the Raman amide I and amide III bands and most of methods for FTIR amide I and II bands were used under Matlab® as a part of our Vibrational Spectroscopy Toolbox & Application [3]. Dependence of informational content and accuracy of the Raman spectra on a protein concentration has been also examined.

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1. D. B. Smith and K. S. Johnson, *Gene*, **67** (1988), 31-40.
2. A. Lobley, L. Whitmore, B. A. Wallace, *Bioinformatics*, **18** (2002), 211-212.
3. V. Kopecký, J. Bok, K. Hofbauerová, In Matlab 2001; J. Uhlíř (Ed.), VŠCHT: Prague (2001), 180-183.

DENSITY MODIFICATION UTILIZING STEREO - CHEMICAL KNOWLEDGE

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Density modification (DM) is a standard routine for improving phases, particularly for structures beyond atomic resolution [1]. For atomic resolution structures the Fourier recycling with peak picking is usually sufficient tool to solve the structure. In cases the phases are not very good, Fourier inversion of modified superposition or electron density maps may be used as the first step of a phase refinement [2, 3]. Density modification using molecular fragments (DMF) offers a tool for strengthening structure determination methods in a way analogous to molecular replacement. Chemical information can be entered into the process of structure determination by 6-D phased rotation and translation function (PZO method) [4].

Fragment electron density is calculated for top peaks of the PZO search. For each peak the fragment atoms are positioned in the unit cell. Electron density is constructed for FFT calculation of structure factors [5]. Electron density is sampled on a uniform grid. Contributions from all fragment atoms are summed at each grid point. An electron density map belonging to one fragment and one PZO peak is created. Maps from different peaks and from different fragments are combined together to form a fragment map. In the process of density modification the fragment map is combined with electron density map. DMF map is modified by omitting low-density (negative) regions. Then structure factors are calculated by reverse FFT. Density modified structure factors (FDMF) are fitted against observed structure factors or normalized structure factors in bins of different resolution. These structure factors can be used for R-factor calculation and for weighting Fourier coefficients. The density modification (DM) is exactly the same procedure as DMF but no fragment map is used. Only low-density regions are omitted.

In the first group of tests organic structures containing sulfur or chlorine atoms were selected. Atomic minimum superposition was not used in the phasing process. Phases calculated from heavy atoms were used for density modification. Phases from heavy atoms were pre-refined by several cycles of DM, where necessary. Results of DMF were compared with results of DM. In every case the DMF is su-

perior do DM. In both methods the cut-off was fixed at 4% of the top electron density.

Comparison with similar calculation on the same structure revealed, that the fragment positioning is an important step in this structure determination or a way of considerable reducing number of Fourier cycles needed to solve the structure.

1. Cowtan, K. D. & Mail, P. *Acta Cryst*. **D49**,(1993) 148-157.
2. Simonov, V. I. Computational Crystallography, edited by D. Sayre, pp. 150-158. Oxford, Clarendon Press.(1982).
3. Shiono, M. & Woolfson, M. M. *Acta Cryst.* **A48**, (1992) 451-456.
4. Pavelcik, F., Zelinka, J. & Otwinowski, Z. *Acta Cryst.* **D58**, (2002) 275-283.
5. Agarwal, R. C. *Acta Cryst*. **A34** (1978) 791-809.

DETAILED STUDY OF INTERACTIONS BETWEEN SMALL MOLECULES AND PROTEINS USING MOLECULAR MODELING

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Detailed knowledge of interactions inside the proteins plays an important role in drug design. Experimental methods such as X-crystallography, NMR spectroscopy and neutron diffraction are typical experimental methods to analyze these interactions at atomic level [1]. These experimental methods can, in some cases, be complemented by molecular modeling methods. The molecular docking combined with flexible conformational search, molecular dynamics and quantum dynamics are the most used modeling methods at this time.

Recently, the interactions of solvent molecules with cyclin dependent kinase (CDK2) using molecular dynamics were studied in our laboratory [2]. The study was extended to molecular dynamics with mixtured solvent (water molecules mixtured with small organic molecules) and also to utilization of systematic molecular docking of small organic molecules into CDK2 using CICADA [3, 4] program. The results of such calculations will be used. As a mixtured solvent water + dimethylether and water + methylamine were used. The results will be compared with systematic docking search combined with molecular dynamics and also steered molecular dynamics method.

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1. G. Koellner, G. Kryger, C. B. Millard, I. Silman, J. L. Sussman & T. Steiner, *J. Mol. Biol.*, **296** (2000) 713 - 735.
2. Z. Kříž, M. Otyepka, I. Bártová & J. Koča, Proteins: Structure, Function and Genetics, (in press).
3. J. Koča & P. H. J. Carlsen, *J. Mol. Struct. (Theochem)*, **308** (1994), 13 - 24.
4. Z. Kříž, J. Koča, A. Imberty, A. Charlot, R. Auzély-Velty, *Org. Biomol. Chem.*, **1** (2003) 2590 - 2595.

COMPUTATIONAL STUDY ON PIGMENTS OF PHOTOSYSTEM II REACTION CENTRES

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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 energy conversion is located in so-called reaction centre (RC).

Recently, structure of the PSII complex isolated from cyanobacteria *Synechococcus elongatus* has been presented at the resolution of 3.8 Å [1] and from *Synechococcus vulkanus* at 3.7 Å [2]. Also time constants of charge distribution and molecules involved in this process are already known, although X-ray and also spectroscopy methods are unfortunatelly not able to give us sufficient explanation of the charge-separation processes. Theoretical molecular modelling study could be such complementary method for a complex understanding of properties and function of the PSII RC pigments during a charge-separation process.

In our last study [3, 4] we have combined the structural homology modelling based model proposed by Svensson et al. [5] (1DOP model), and the experimental structure presented by Zouni et al. [1] and succesfully calculated absorption and circular dichroism spectra using point-dipole approximations [6] and compared them with the experimental results in order to locate accumulated chlorophyll cation during a light treatment of Photosystem II reaction centre in presence of artificial electron acceptor silicomolybdate. Along with the spectra calculations the charge distribution on the primary electron acceptor pheophytine of the combined model in a ground and reduced state and its influence on the surrounding protein environment is studied by using quantum chemistry methods on a semiempirical (ZINDO-1), density functional (B3LYP) and ab initio (HF) levels.

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1. Zouni, A., Witt, H.T., Kern, J., Fromme, P., Krauss, N., Saenger, W. and Orth, P. *Nature* **409** (2001) 739-743.
2. Kamiya N. and Shen J.-R. *Proc. Natl. Acad. Sci. USA* **100** (2003) 98-103.
3. Vácha F., Kutý M., Durchan M., Šiffel P. and Pšenčík J.: Localisation of accumulated chlorophyll cation in reaction centre of photosystem II. *Materials Structure* **10** (2003) No.1, 21-25.
4. Vácha F., Kutý M. and Pšenčík J. Experimental and calculated optical spectra as a tool for chlorophyll cation localization in reaction centre of photosystem II. The 21st European Crystallographic Meeting, Durban, SA, 24-29 August 2003.
5. Svensson, B., Etchebest, C., Tuffery, P., van Kan, P., Smith, J. and Styring, S. *Biochemistry* **35** (1996) 14486-14502.
6. Durrant, J.R., Klug, D.R., Kwa, S.L.S., van Grondelle, R., Porter, G. and Dekker, J.P. *Proc. Natl. Acad. Sci. USA*, **92** (1995) 4798-4802.

STRUCTURAL STUDIES OF ANTI-CA IX MONOCLONAL ANTIBODY M75 Fab FRAGMENT IN COMPLEX WITH ITS EPITOPE PEPTIDE

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CA IX (carbonic anhydrase IX) is a cell surface protein, associated with several types of human carcinomas[1]. It is almost 100 % associated with cervical carcinomas, carcinomas of oesophagus and with renal clear cell carcinomas, while it is absent in normal tissues of corresponding organs. This suggests that CA IX gene represents a novel oncogene [2]. CA IX exerts capacity of binding to cell surface receptors [1]. It is a member of the alpha class of carbonic anhydrases - zinc metalloenzymes that catalyze the reversible conversion between CO_2 and bicarbonate.

Monoclonal antibody M75 [3] kindly provided by Dr. Závada reacts excellently with CA IX. Using synthetic oligopeptides, the epitope of mAb M75 was localized in the proteoglycan-like domain of CA IX and identified as six times repeating amino acid sequence PGEEDLP [1]. Structural studies of the M75 Fab fragment in complex with its epitope peptides should be helpful in rational drug design of compounds suitable for use in human oncology.

M75 mAb was isolated from TC medium of hybridoma cells M75 using Protein A Sepharose. Fab fragment of M75 antibody was obtained by controlled papain digestion of IgG M75 and further purification on DEAE Sephacel. Purity of the product was assessed using gel filtration, SDS PAGE and isoelectric focusing.

Using vapor diffusion hanging drop technique we obtained crystals of free Fab M75 and crystals of Fab M75 in complex with epitope peptide PGEEDLPGEEDL. Datasets for both crystals were collected at 150 K using in-house diffractometer (Nonius FR 591 generator, 345mm MarResearch Image Plate detector). Crystals belong to triclinic spacegroup P1. The Fab M75 structure was solved by molecular replacement, using Fab Bv04-01 (PDB code 1NBV) as search model. Probably due to high mobility of the variable loops the structure of free Fab M75 could not be refined. However structure of Fab M75 in complex with the epitope peptide was successfully refined at 2.0 Å resolution (the final R- and free R factors are 0.194 and 0.260 respectively).

Only the first eight N-terminal residues of the peptide (PGEEDLPG) are visible in electron density maps. The C-terminal residues extend out from the antigen-binding site and are most probably flexible. The interactions between Fab M75 and epitope peptide include extensive van der Waals contacts and several hydrogen bonds. All six CDRs of Fab M75 are involved in contacts with antigen. With the exception of the third peptide residue (E), which points towards the exterior of the antigen binding groove the remaining residues from the peptide establish several contacts. Due to shift of the variable H3 loop in direction to the light chain CDRs the structure displays an interesting and unusual mode of epitope binding which involves van der Waals interactions between epitope peptide and the N terminal end of the Fab heavy chain.

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1. Závada J, Závadová Z, Pastorek J, Biesová Z, Ježek J, Velek J., *Br J Cancer.* **82** (2000) 1808-1813.
2. Závada J, Závadová Z., Pastoreková S., Ciampor F., Pastorek J., Zelník V., *Int. J. Cancer* **54** (1993) 268-274.
3. Pastoreková S, Závadová Z, Koš ál M, Babušíkova O, Závada J., *Virology* **187** (1992) 620-626.

ARCHITECTURE OF THE MEMBRANE FORMS OF NKR-P1 RECEPTORS, AND THEIR SIGNALING COMPLEXES IN RAT NK CELL MEMBRANE MICRODOMAINS

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Natural killer (NK) cells are cytotoxic effector lymphocytes which do not rearrange or express antigen-specific surface receptors [1]. Although many NK cell receptors that transduce signals leading to activation or inhibition of natural killing have been identified, the details of their membrane topology and physiology remain poorly understood. In T-lymphocytes, many components of the activation receptor complexes are concentrated in glycolipid-enriched membrane rafts, which play important role in the membrane activation synapse [2]. However, although morphological studies revealed movement of plasma membrane rafts into the area of contacts between NK cells and their sensitive targets [3], no detailed characterization of the membrane receptor complexes has been provided in NK cells.

We have used fresh sorted rat NK cells, and variants of the rat NK leukemic cell line RNK-16 as model systems to study the membrane environment of NKR-P1 receptor, a key activation antigen of rat NK cells [4]. Studies with the bacterially expressed monomeric NKR-P1 receptor isoforms revealed important differences in the recognition of simple and complex carbohydrate ligands. The ability of carbohydrate dendrimers to precipitate recombinant dimeric forms of NKR-P1 receptors opened the way for studies of the native forms of these receptors isolated from their membrane microdomains. Carbohydrate dendrimers interact specifically with NK cells mainly through NKR-P1A isoform. They proved to be specific activators of NK cells mediating the increase in intracellular calcium, generation of inositol phosphates, and natural killing [5]. Blue native electrophoresis revealed the native form of NKR-P1A receptor as a part of large 220 kDa protein complex. Moreover, a shotgun strategy based on microcapillary HPLC in combination with tandem mass spectrometry allowed us to study the complete protein profile of the NKR-P1$^+$ membrane microdomain. We identified large sets of proteins associated with these microdomains but their detailed composition depended on the chemical environment used during the isolation. These proteins included additional NK cell receptors (CD2, CD18, CD44, CD45, membrane adaptors and signaling enzymes (LAT, N-TAL, lck, ras, rab), effector enzyme complexes (ATP synthase, VDAC proteins, annexins) as well as the proteins of the cytoskeleton scaffold (actin, clathrin, cofillin, ezrin, flotillin) [6].

Functional and structural characteristics of the individual protein-lipid complexes defining the molecular architecture of the entire microdomain are currently under active investigation in our laboratory.

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1. L. L. Lanier, *Annu.Rev.Immunol*., **16** (1998) 359-396.
2. K. Simons & D. Toomre, *Nat.Rev.Mol.Cell.Biol*., **1** (2000) 31-41.
3. M. S. Fassett, D. M. Davis, M. M. Valter, G.B. Cohen, J. L. Strominger, *Proc.Natl.Acad.Sci.USA* **98** (2001) 14547-52.
4. K. Bezouška, *J.Biotechnol*, **90** (2002) 269-290.
5. O. Plíhal, J. Sklenář, et al., unpublished.
6. P. Man, P. Novák, M. Cebecauer, A. Fišerová, K. Bezouška, manuscript in preparation.

NMR DISTANCE RESTRAINTS AT LOW TEMPERATURES - AN APPLICATION TO A DNA HAIRPIN

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Short DNA hairpins play a significant role in a number of biological processes. The most interesting feature of the oligonucleotides with a general sequence d(GCGNAGC) (N = A, G, C, T) is their extraordinary stability represented by high melting temperatures, polyacrylamide gel mobility and resistance against nucleases. Detailed knowledge of these structures helps understand their unique behavior.

The d(GCGAAGC) structure has been solved previously by NMR spectroscopy using the NOE-derived distances, torsion angles and residual dipolar couplings [1]. The aim of the present study is a new structure calculation based on NOE data measured at temperatures below 273 K when the intramolecular motions and the chemical exchange of the amino protons are inhibited. Compared to the studies at the ambient temperature, this approach allows us to extract more distance restraints for the use in the molecular dynamics calculations.

A series of the 2D-NOESY spectra with the mixing times in a range of 50 to 400 ms has been measured at 268K. The assignment of the spectra revealed 11 of 14 assigned amino proton resonances compared to 6 of 14 resonances extracted at 298K. 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. Our treatment of the NOESY cross-peak volumes includes both the Isolated

Spin Pair Approach (ISPA) and the relaxation matrix approach represented by the MARDIGRAS and MORASS algorithms in order to account for the spin diffusion. The choice of the method for the extraction of a sufficiently large number of accurate proton distances from 2D NOE cross-peak intensities may be important for the subsequent structure calculations. Here we compare the NOE-derived interproton distances obtained by the ISPA and the relaxation matrix analyses for several sets of experimental data.

1. P. Padrta, R. Štefl, L. Králík, L. Žídek, V. Sklenář, *J. Biomol. NMR*, **24**, (2002), 1 - 14.

2. U. Schmitz, D. A. Pearlman, T. L. James, *J. Mol. Biol*., **221**, (1991), 271 - 292.

MODELING OF THE VIBRATIONAL CIRCULAR DICHROISM OF SOLVATED PEPTIDES

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Vibrational circular dichroism (VCD) spectra of newly synthesized short proline-containing peptides Tyr-Asp-Pro-Ala-(Pro)N have been measured, in order to elucidate there solution geometries. We are developing a theoretical model for spectra simulations, based on complex molecular dynamics and ab initio DFT calculations. Structures of the peptide and the water layer are obtained by molecular dynamic, with the Amber force field. Then the peptide and closest water molecules are partially optimized, using the restricted normal technique, with the ONIOM method implemented in Gaussian 03, at the PM3/BPW91/6-31G** level. This new method should provide more realistic models that the continuum solvent models used previously [1]. The calculated infrared absorption and VCD spectra profiles are sensitive to the hydration and conformation of the peptide and reasonably well reproduce the experimental intensities.

1. Bour, P.; Kubelka, J.; Keiderling, T. A. *Biopolymers*, **65**, (2002), 45-69.

NMR STUDY OF PACE11 FROM THE HYPERTHERMOPHILIC ARCHAEON *PYROCOCCUS ABYSSI*

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The analysis of the hyperthermophilic archaeon *Pyrococcus abyssi* genome showed that some proteins are highly conserved between Archaea and Eukaryotes. 32 proteins have been listed and called the PACE proteins (Proteins of Archaea Conserved in Eukaryotes) [1]. One of these proteins, PACE11, we decided to study by NMR. This protein has been biochemically characterized as a monomeric phosphopantetheine adenylyltransferase (PPAT) involved in the Coenzyme A (CoA) biosynthesis pathway [2]. This pathway, consisting of 5 steps, has been described in Bacteria and Eukaryotes but not in Archaea. PPAT is involved in 4th step of the CoA biosynthesis, catalyzing the reversible transfer of an adenylyl group from ATP to 4'-phosphopantetheine giving dephosphoCoA (dPCoA). So far the 3D structure of only a bacterial PPAT (from *E. coli* and *T. Thermophilus*) has been solved [3]. PACE11 shows very low sequence identity with these bacterial proteins.

PACE11 project is to get structural and dynamical information of this protein. PACE11 is PPAT of *Pyrococcus abyssi*, which is composed of 157 residues. In order to get enough protein for the NMR study a synthetic gene of PACE11 (having no *E. coli* rare codons) was cloned into the pET28a plasmid and the expression of the gene was tested showing a very good production of PACE11. A ^{15}N sample was then prepared and the 1H-15N HSQC recorded on this sample showed that PACE11 is well folded.

1. Matte-Tailliez O, Zivanovic Y, Forterre P. *Trends Genet* **12** (2000), 533-536.

2. Armengaud J, Fernandez B, Chaumont V, Rollin-Genetet F, Finet S, Marchetti C, Myllykallio H, Vidaud C, Pellequer JL, Gribaldo S, Forterre P, Gans P., *J Biol Chem* **278**, (2003) 31078-31087.

3. Izard T. *J Mol biol* **315** (2002), 487-495.

ANALYSIS OF INTERNAL MOTION FROM MOLECULAR DYNAMICS OF MAJOR URINARY PROTEIN-I

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Investigation of the protein of mouse urine has shown that it consists predominantly of a group of closely related proteins termed the Major Urinary Proteins (MUPs). These are acidic proteins (pI values from 4.2 to 4.7) with molecular masses of approximately 19 kDa. The MUPs are associated with pheromonally active ligands including relatively tightly bound 2-sec-butyl-4,5-dihydrothiazole. Thus MUPs may serve as a model of proteins binding small, hydrophobic ligands that are known to possess the capability of chemical signaling. Structure of MUP-I was determined crystallographically.

The X-ray structures of MUP-I with and without ligand were taken and hydrogens, counter-ions and box of explicit water molecules were added. The system was minimized, heated and equilibrated and then the molecular dynamics was ran. From whole trajectory a 6ns-long part was taken and used for further analysis of internal motions of MUP-I. Correlation functions and frequency dependent order parameters have been calculated.All molecular dynamics was performed with AMBER7 software package.

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STRUCTURAL INVESTIGATION OF DISTINCT SPECIFICITY OF TWO ISOFORMS OF RAT RECEPTOR NKR-P1

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Receptor NKR-P1 is one of lectin-like proteins on the surface of rat lymphocytes [1]. Unique isoforms of this receptor can have both activating and inhibitory function in the process of killing of tumor or virus-infected cells. The isoform NKR-P1A is activating receptor, whereas the isoform NKR-P1B has an inhibitory activity. However, the primary sequence of both proteins is very similar. We can conclude from the results of binding experiments with recombinant extracellular portions of both isoforms, that monosacharide specificity of these proteins are the same. On the other hand, we can observe a principal difference if we compare the results of binding experiments with oligosaccharides and glycodendrimers [2]. We use computer modelling to suggest structural basis of such diversity and try to find possible functional implications.

1. Yokoyama W. M. *Curr. Biol.*, **5** (1995) 982.
2. Plíhal O. et al., *Collection Czech Chem. Commun.*, in press.

IDENTIFICATION OF PROTEINS ASSOCIATED WITH LIPID RAFTS OF JURKAT T-CELL LINE BY MASS SPECTROMETRY

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The aim of our study is to develop a method for complete characterization of proteins associated with lipid rafts of Jurkat T-cell line. Lipid rafts are rich in cholesterol and sphingolipids. Due to the enrichment of cholesterol and sphingolipids in the lipid rafts, they concentrate in low density fractions during the sucrose density gradient ultracentrifugation. Upon cellular activation, signal transduction molecules (*src*-kinases *lck* and *fyn*, CD45 antigen, and others) concentrate in lipid rafts, and initiate polymerization of cytoskeletal proteins leading to the formation of an immune synapse.

While conventional immunochemical staining can reveal only one protein in a single experiment, sensitive and robust mass spectrometric technique provides us with more detailed information on protein content of the lipid raft. We applied a more rigorous purification scheme involving isolation of plasma membranes after hypotonic lysis to exclude the "non-plasmatic" membrane microdomains. Moreover, we used two types of lysis buffers. TNE with 1% NP-40 represents classical extraction used in lipid raft studies. The "non-classical" MES buffer with Na_2CO_3 allows removal of non-specifically associated proteins. Proteins were identified by two approaches: immunoblot with ECL detection, and microcapillary liquid chromatography in combination with electrospray ionization tandem mass spectrometry (microHPLC-ESI-MS/MS).

By applying these conditions, we have identified a large number of functionally relevant proteins involved in signaling, cytoskeletal association, cellular adhesion and other processes. Western blot analysis has revealed typical GEMs proteins (Lck and CD59) and thus verified the quality of sample preparation. On the other hand mass spectrometry has found not only GEMs markers but also many other molecules involved in cell signaling processes like Ras-related protein, GTP-binding protein, S100-Ca^{2+} binding proteins, CD45, 4F2, HLA-I, etc.

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MOLECULAR DYNAMICS STUDY OF NOVEL RNA-RNA INTERACTIONS MOTIFS: RNA KISSING COMPLEXES

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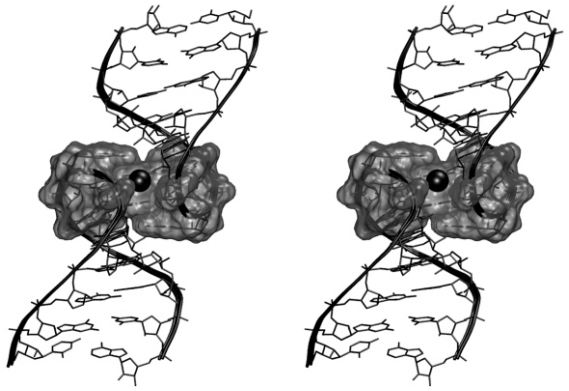

Explicit solvent molecular dynamics (MD) simulations were carried out for three RNA kissing-loop complexes. The theoretical structure of two base pairs (2 bp) complex of H3 stem-loop of Moloney murine leukemia virus agrees with the NMR structure with modest violations of few NMR restraints comparable to violations present in the NMR structure. In contrast to the NMR structure, however, MD shows relaxed intermolecular G-C base pairs.

The core region of the kissing complex forms a cation-binding pocket with highly negative electrostatic potential. The pocket shows nanosecond-scale breathing motions coupled with oscillations of the whole molecule. Additional simulations were carried out for 6 bp kissing complexes of the DIS HIV-1 subtypes A and B. The simulated structures agree well with the X-ray data. The subtype B forms a novel four-base stack of bulged-out adenines. Both 6 bp kissing complexes have extended cation-binding pockets in their central parts. While the pocket of subtype A interacts with two hexacoordinated Mg^{2+} ions and one sodium ion, pocket of subtype B is filled with a string of three delocalized Na+ ions with residency times of individual cations 1-2 ns. The 6 bp complexes show breathing motions of the cation-binding pockets and loop major grooves.

A STRUCTURAL MODEL OF HUMAN MT2 MELATONIN RECEPTOR AND ITS MELATONIN RECOGNITION SITE

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(see page 12 for full paper)

MEMBRANE TRANSPORT WITHOUT RECEPTORS?

THE ROLE OF CYCLOSPORINES AND SILYMARINES STRUCTURES FOR THEIR INTERACTIONS WITH LIPIDS OF HEPATOCYTE PLASMA MEMBRANE

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(see page 15 for full paper)

MOLECULAR DYNAMICS SIMULATIONS ON GLYCOSYLTRANSFERASE LgtC

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The glycosyltransferases catalyze the transfer of glycosyl moieties from a donor sugar to an acceptor. In most cases, the donor is a nucleoside phosphosugar and the acceptor a hydroxyl group of another sugar, a lipid, or another component of glycoconjugates. The catalytic mechanism of the glycosyltransferases, however, still remains a mystery. The glycosyltransferases are classified as either retaining or inverting, depending on the stereochemical outcome of the reaction catalyzed.

The galactosyltransferase LgtC [1] [2] from *Neisseria meningitidis* is a retaning glycosyltransferase catalyzing a key step in the biosynthesis of lipooligosaccharide structure by transferring a-D-galactose from UDP-galactose to a terminal lactose. The comprehension of the catalytic mechanism is an essential precondition to be able to design an effective inhibitor of this enzyme, and to find an effective drug against the bacterial pathogen in this way. We report here the first molecular dynamics simulations of LgtC in water environment and in the presence of its substrates. The analyses of the trajectories provide a first insight on the catalytic mechanism of this glycosyltransferase.

The starting coordinates were taken from the X-ray structure of LgtC galactosyltransferase with donor and acceptor sugar analogs, which is deposited in the Protein Databank under the code 1GA8. The original pdb file had to be modified, as it contained coordinates for 278 amino acids, the donor analog UDP-2-deoxy-2-fluorogalactose and the acceptor analog 4-deoxylactose. The four missing

residues 218-221 were added in Sybyl 6.7 using protein loop search. The donor analog was replaced with UDP-galactose (native donor), and the acceptor analog was replaced with lactose (native acceptor).

Simulations were performed using the AMBER-6.0 program package with several new parameters especially developped for nucleotide sugars [3]. All simulations were run with the SANDER. The MD trajectories were analyzed with the CARNAL and PTRAJ.

Four MD simulations were run using the X-ray derived geometry as the starting point. The mobility of two loops essential for the correct functioning of the enzyme were studied together with the stability of coordination of the manganese ion and its role in the binding of substrates.

1. Persson, K., et al., Crystal structure of the retaining galactosyltransferase LgtC from Neisseria meningitidis in complex with donor and acceptor sugar analogs. *Nature Structural Biology*, 2001. **8**(2) p. 166-175.
2. Ly, H.D., et al., Mechanistic Studies of a Retaining a-Galactosyltransferase from Neisseria meningitidis. *Biochemistry* 2002, 2002. **41** (16) p. 5075-5085.
3. Petrová, P., J. Koča, and A. Imberty, Potential Energy Hypersurfaces of Nucleotide Sugars: Ab Initio Calculations, Force-Field Parametrization, and Exploration of the Flexibility. *J. Am. Chem. Soc*., 1999. **121** (23) p. 5535-5547.

ISOLATION AND CRYSTALLISATION OF THE KEY PROTEIN IMPORTANT IN THE REDOX CONTROL OF *Streptomyces coelicolor*

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Thioredoxins are ubiquitous proteins that serve as reducing agents and general protein disulfide reductases in living cells. Thioredoxin together with thioredoxin reductase and coenzyme NADPH formed the cellular redox enviroment in the *Streptomyces* [1]. *Streptomyces* are Gram-positive spore-forming soil bacteria with specific life cycle. *Streptomyces coelicolor* is geneticaly best known representative of the genus. It is suitable model for study of the proteins important in thiol-disulfide status in the cell because glutathione in not made by actinomycetes.

Thioredoxins belong to the structural family that includes glutaredoxin, glutathion peroxidase, bacterial protein disulfide isomerase and N-terminal domain of glutathione transferase [2]. The primary structure of many thioredoxins are known, and show 27-69 % sequence identity. 3D structure of thioredoxins from a number of species, including man [3] and *Escherichia coli* [4] are known, too. In all these 3D structure thioredoxin is a compact globular protein with a five-stranded ß sheet surrounded by four ? helices.

In our work we over-expressed thioredoxin 1 of *S. coelicolor* His.Taq fusion protein in *Escherichia coli*. The protein was purified using single step metal chelate affinity chromatography. Homogenous protein was used for study of the protein stability, its role in the redox control in the cell and the control of protein - protein interaction. Purified protein was used for crystallization trials, too. The crystallization conditions for thioredoxin 1 of *S. coelicolor* was determinated and we have obtained the crystals suitable for x-ray diffraction analyses. The premilitary x-ray study has showed that protein crystallized in P222 space group with cell parameters 33.2, 43.5, 143.9, 90.0, 90.0, 90.0.

1. M.S. Paget, V. Molle, G. Cohen, Y. Aharonowitz, M.J. Buttner, *Mol. Microbiol.* **42** (2001) 1007-20.
2. J.L. Martin, *Structure* **3 (**1995) 245-250.
3. A. Weichsel, J.R. Gasdaska, G. Powis, W.R. Montfort, *Structure* **4 (**1996) 735-51.
4. S.K. Katti, D.M. LeMaster, H. Eclund, *J. Mol.Biol.* **212** (1990) 167-184.

CLONING, EXPRESSION AND CHARACTERIZATION OF THE MYCOBACTERIAL HALOALKANE DEHALOGENASES Rv2296 AND Rv2579

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Haloalkane dehalogenases are enzymes catalyzing cleavage of a carbon-halogen bond of halogenated compounds using a hydrolytic mechanism. The products of the reaction are a halogen ion and a corresponding alcohol. Known sequences of genes encoding haloalkane dehalogenases *dhlA, dhaA* and *linB* were compared with all sequences accessible in genetic databases. This comparison revealed a presence of two genes coding for putative haloalkane dehalogenases in the genome library of *Mycobacterium tuberculosis* H37Rv. Identified genes were *rv2296* and *rv2579* [1]. We have used genetically closely related *Mycobacterium bovis* 5032/66 izolated from the cattle to clone the genes of putative mycobacterial dehalogenases.

Gene *rv2296* was amplified by PCR reaction using specific primers. PCR amplicon of the gene *rv2296* was cloned into expression vector pCR T7/NT- TOPO and the ligation mixture was transformed to *Escherichia coli* OneShot TOP10F competent cells. The *rv2296* gene was sequenced from both sides to ensure accuracy. Expression of *Rv2296* protein was performed in bacterial cells *E. coli* BL21 (DE3)LysS. Solubility of the protein was supported using different conditions for preparation of crude extract. Active

purified protein Rv2296 could not be prepared using the standard purification protocols. The substrate specificity of crude extract Rv2296 was tested towards thirty-four different halogenated substrates.

Gene *rv2579* was amplified by PCR reaction using specific primers, which introduced hexahistidyl tail to C-terminus of the protein. PCR amplicon of the gene *rv2579* was cloned into pUC18 vector and sequenced. For expression of Rv2579, the gene was re-cloned from pUC18 to pAQN. *E. coli* BL21 was used as the host strain for expression of Rv2579 protein. Expressed protein was purified to homogeneity using immobilized metal affinity chromatography with the yield 5 mg of a protein per 1 L of a bacterial culture. Protein Rv2579 was tested for temperature- and pH-optimum. The highest activity of protein Rv2579 was observed at 45 °C. Protein showed double pH optimum (pH 5.5 and pH 8.5). The effect of storage temperature and the effect of stabilizing additives on protein was also determined.

1. Jesenská A., Sedláček I., Damborský J. Dehalogenation of Haloalkanes by Mycobacterium tuberculosis H37Rv and Other Mycobacteria. *Applied and Enviromental Microbiology* **66**(2000): 219-222.

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CRYSTALLIZATION OF THE NOVEL FLAVODOXINE-LIKE PROTEIN, WrbA, - ON THE WAY TO THREE-DIMENSIONAL STRUCTURE

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Keywords: flavodoxine-like protein; tryptophan-repressor binding protein; WrbA apoprotein; WrbA holoprotein; FMN; standard crystallization techniques; advanced crystallization techniques

Tryptophan (W)-repressor binding protein A, WrbA, identified as an *E. coli* stationary-phase protein was named for its reported effect on the interaction between tryptophan repressor and DNA [1]. Later work [2] showed that this effect was non-specific, leaving the physiological role of WrbA unknown. According to sequence analysis and homology modeling [3] WrbA was identified as the founding member of a new family of flavodoxin-like proteins, which displays low but structurally significant sequence similarity with the flavodoxins. The members of WrbA family are predicted to share the open, twisted a/b flavodoxin fold, but with a short conserved insertion unique for the new family. This structure motif could account for experimental observations that some family members are dimeric in solution, including also finding that WrbA creates a dimer-tetramer equilibrium at micromolar concentrations [2]. Unlike typical flavodoxins [4], these proteins bind FMN relatively weakly but still specifically. The computer analysis [3] indicated some structural differences in the flavin-binding pocket, which may explain the lower affinity of WrbA for FMN. Due to these peculiarities the structural analysis may aid in understanding the physiological roles of WrbA family members. These factors motivated our research for diffraction-quality crystals.

Purified WrbA apoprotein and holoprotein were used for crystallization trials. Standard and advanced crystallization techniques were applied to crystallize mentioned proteins. WrbA apoprotein crystals grown in capillaries were measured directly at synchrotron DESY (beamline X13) in Hamburg (Germany). Crystals diffracted to a resolution of 2.2 Å. Attempts with variable growing conditions are performed to improve quality of apo- and holoprotein crystals.

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1. Yang, W., Ni, L. & Somerville, R.L., *Proc. Natl. Acad. Sci.*, USA **90**(1993), 5796-5800.
2. Grandori, R., Khalifah P., Boice J.A., Fairman, R., Giovanielli, K. & Carey, J., *J. Biol. Chem.*, **273** (1998), 20960-20966.
3. Grandori, R. & Carey, J., *Trends Biochem. Sci*., **19**(1994), 72.
4. Burnett, R.M., et al., *J. Biol. Chem.*, **249**(1974), 4383-4392.

MOLECULAR DYNAMICS STUDY OF CIS-ACTING HEPATITIS DELTA VIRUS RIBOZYME: NON-WATSON-CRICK BASEPAIRS AND SPECIFIC HYDRATION SITES

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The hepatitis delta virus (HDV) ribozymes are self-cleaving RNA sequences critical to the replication of small RNA genomes. The cleavage activity of the genomic and antigenomic ribozymes can be mapped to minimal ~85 nt motifs. Molecular dynamics simulations of the cleavage product form of genomic cis-acting HDV ribozyme were performed with explicit inclusion of solvent and counterions. Simulation of the native structure with protonated C41 residue was carried out for 15 ns, and exhibited stable trajectories retaining all Watson-Crick and non-Watson-Crick basepairs, except fluctuations and transient disruptions at specific sites. Three additional simulations were carried out for ~45 ns in all to clarify the structural and dynamic effect of selected mutations. In one of them C41 was left unprotonated. Significant local rearrangements occur during this simulation, such as irreversible disruption of C41·C73 basepair as well as rearrangement of nucleotides close to the catalytic pocket, thus confirming N3-protonation of C41 in the native molecule. The most considerable event is rotation of G76 residue and formation of numerous hydrogen binding interactions of the base of G76 inside the catalytic pocket, which are not present in the crystal structure. However, the prolongation of this simulation after protonation of C41 base did not result in recovery of the original structure of disrupted non-Watson-Crick basepairs as well as extrusion of G76 outside the catalytic pocket. This fact is an evidence of significant height of energy barrier between considered conformational states and gives rise to the question about a sufficiency of sampling in both simulations. One of the commonly considered self-cleavage reaction mechanism include C75(N+) as a general acid catalyst. The simulation included protonated C75 was performed to obtain insight into the possible protonation state of C75 base at the product form of the ribozyme under investigation. In this simulation interaction of C75 with cleavage site occurred unstable and irreversibly broke after 1 ns simulation. Therefore, C75 is not likely to be protonated at the ribozyme product form.

The simulation revealed several critical, highly ordered hydration sites with close to 100% occupancies and long residency times of individual water molecules which form water bridges in non-Watson-Crick base pairs or participate in formation of catalytic pocket environment. Sodium cation coordination sites with occupancies above 50% were also found.

Forthcoming meetings

European Powder Diffraction Conference

Prague, Czech Technical University

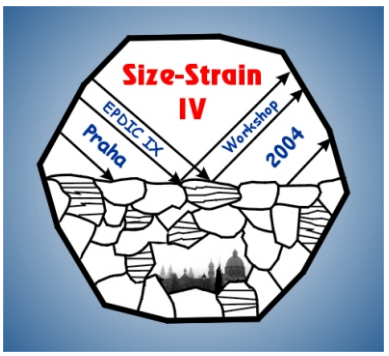
September 2 – 5, 2004

7th Biennial Conference

on High Resolution X–Ray Diffraction and Imaging

September 7 – 10, 2004

Congress Center Průhonice near Prague



Size - Strain IV

Prague, Czech Technical University

August 31 - September 2, 2004