

Lectures - Friday, March 19, afternoon, Session V**L21****STRUCTURE OF THE 14-3-3/FOXO4 COMPLEX DERIVED FROM THE FLUORESCENCE SPECTROSCOPY DATA****T. Obšil^{1,2}**¹*Faculty of Science, Charles University in Prague, Hlavova 8, 12843 Prague 2, Czech Republic*²*Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague 4, Czech Republic
obsil@natur.cuni.cz*

The FOXO class of forkhead transcription factors consists of four members (FOXO1, FOXO3, FOXO4, and FOXO6) that play a central role in cell cycle control, differentiation, metabolism control, stress response, and apoptosis [1]. Transcriptional activity of FOXO proteins is regulated through the insulin-phosphatidylinositol 3-kinase-AKT/protein kinase B (PKB) signaling pathway. The AKT/PKB-mediated phosphorylation triggers phosphorylation of additional sites by casein kinase-1 and dual specificity tyrosine-regulated kinase-1A and induces FOXO binding to the 14-3-3 protein. The role of 14-3-3 proteins in the regulation of FOXO forkhead transcription factors is at least 2-fold. First, the 14-3-3 binding inhibits the interaction between the FOXO and the target DNA. Second, the 14-3-3 proteins prevent nuclear reimport of FOXO factors by masking their nuclear localization signal [2]. The exact mechanisms of these processes are still unclear, mainly due to the lack of structural data. We used fluorescence spectroscopy to investigate the mechanism of the 14-3-3 protein-dependent inhibition of FOXO4 DNA-binding properties. Time-resolved fluorescence measurements revealed that the 14-3-3 binding affects fluorescence properties of 5-(((acetylamino)ethyl)amino) naphthalene-1-sulfonic acid moiety attached at four sites within the forkhead domain of FOXO4 that represent important parts of the DNA binding interface. Observed changes in 5-(((acetylamino)ethyl)amino)naphthalene-1-sulfonic acid fluorescence strongly suggest physical contacts between

the 14-3-3 protein and labeled parts of the FOXO4 DNA binding interface. The 14-3-3 protein binding, however, does not cause any dramatic conformational change of FOXO4 as documented by the results of tryptophan fluorescence experiments. To build a realistic model of the 14-3-3/FOXO4 complex, we measured six distances between 14-3-3 and FOXO4 using Förster resonance energy transfer time-resolved fluorescence experiments. The model of the complex suggests that the forkhead domain of FOXO4 is docked within the central channel of the 14-3-3 protein dimer, consistent with our hypothesis that 14-3-3 masks the DNA binding interface of FOXO4 [3].

1. A. van der Horst, B.M.T. Burgering, *Nat. Rev. Mol. Cell Biol.*, **8**, (2007), 440.
2. T. Obsil, V. Obsilova, *Oncogene*, **27**, (2008), 2263.
3. J. Silhan, P. Vacha, P. Strnadova, J. Vecer, P. Herman, M. Sulc, J. Teisinger, V. Obsilova, T. Obsil, *J. Biol. Chem.*, **284**, (2009), 19349.

This work was supported by Grant Agency of the Academy of Sciences of the Czech Republic Grant IAA501110801; Grant Agency of the Czech Republic Grant 204/06/0565; Ministry of Education, Youth, and Sports of the Czech Republic Research Projects MSM0021620857 and MSM0021620835 and Centre of Neurosciences LC554; and Academy of Sciences of the Czech Republic Research Project AV0Z50110509.



L22

STRUCTURAL AND MOLECULAR MECHANISM FOR AUTOPROCESSING OF MARTX TOXIN OF VIBRIO CHOLERAE AT MULTIPLE SITES

K. Prochazkova¹, L. A. Shuvalova^{2,3}, G. Minasov^{2,3}, Z. Voburka⁴, W. F. Anderson^{2,3}, K. J. Satchell¹

¹Dep. of Microbiology-Immunology, Feinberg School of Medicine, ²Center for Structural Genomics of Infectious Diseases Medicine, Northwestern University, Chicago, Illinois 60611, USA

³Department of Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA

⁴Institute of Organic Chemistry and Biochemistry, Acad. Sci. Czech Rep. Prague 161-10 Praha 6 katerina.prochazkova@uochb.cas.cz

The *Vibrio cholerae* MARTX toxin is associated with rounding of eukaryotic cells through destruction of the actin cytoskeleton by covalent cross-linking of actin and inactivation of Rho GTPases [1]. The effector domains responsible for these activities are here shown to be independent proteins released from the large toxin by autoproteolysis catalyzed by an embedded cysteine protease domain (CPD) [2]. The CPD is activated upon binding inositol hexakisphosphate (InsP₆), which is present predominantly in eukaryotic cells. We demonstrated that InsP₆ is not simply an allosteric cofactor, but rather binding of InsP₆ stabilized the CPD structure, facilitating formation of the enzyme-substrate complex. The 1.95-Å crystal structure of this InsP₆-bound unprocessed form of CPD was determined and revealed the scissile bond Leu(3428)-Ala(3429) captured in the catalytic site. Upon processing at this site, CPD was converted to a form with reduced affinity

for InsP₆, but was reactivated for high affinity binding of InsP₆ by cooperative binding of both a new substrate and InsP₆. This allowed CPD to cleave MARTX toxin between cytopathic domains by hydrolyzing specifically at Leu-Xaa bonds. Thus we uncovered the mechanism of MARTX autoprocessing, which results in release of cytopathic domains from large holotoxin to reach their targets within eukaryotic cells.

1. K. J. Satchell, *Infect Immun.*, **75**, (2007), 5079.
2. K. Prochazkova, L. A. Shuvalova, G. Minasov, Z. Voburka, W. F. Anderson, K. J. Satchell, *J Biol Chem.*, **284** (2009), 26557.

This work was supported in part by a development project from National Institute of Health Grant U54 AI057153 to Great Lakes (Region V) Regional Center for Excellence in Biodefense and Emerging Infectious Diseases Research.

L23

HUMAN CARBONIC ANHYDRASE COMPLEXES WITH ISOQUINOLINE INHIBITORS

P. Mader¹, J. Brynda¹, R. Gitto², A. Chimirri²

¹Institute of Molecular Genetics AS CR, Prague, mader@img.cas.cz

²Dept. Farmaco-Chimico, Università di Messina, Messina, Italy

Human carbonic anhydrases (CAs) form a family of 14 zinc-containing enzymes that catalyze rapid conversion between carbon dioxide and bicarbonate. This reaction plays role in many physiological processes, such as respiration, regulation of pH and many other biological processes requiring carbon dioxide or bicarbonate. Human isozyme CA II is a cytosolic enzyme and belongs to the most studied isoforms. It is traditionally purified from red blood cells but it has a wide tissue distribution and is found in various organs and cell types [1]. CA II deficiency is associated with osteoporosis, renal tubular acidosis, and cerebral calcification. The transmembrane isoform CA IX has been shown to be linked with carcinogenesis. CA IX is physiologically expressed in the epithelia of the gastrointestinal tract, however it is expressed ectopically in carcinomas derived from kidney, lung, cervix, uteri, oesophagus, breast, and colon. This isoform has been shown to be strongly over-expressed in hypoxic tumors, where it participates in tumor cell envi-

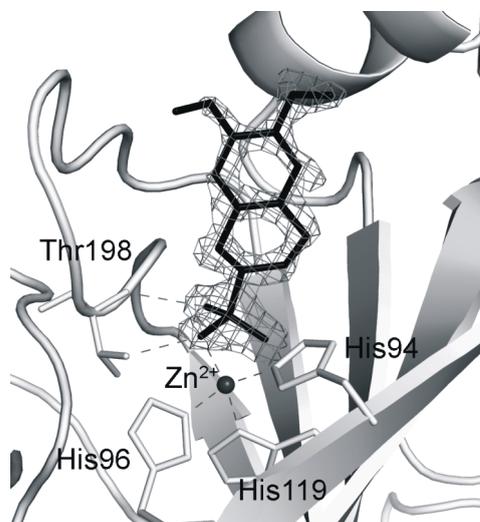


Figure 1. Isoquinoline-sulfonamide derivative binding to active site of human CAII.

onment acidosis and contributes to malignant progression and poor treatment outcome. Designing isoform-selective inhibitors could thus provide potent therapeutics namely for treatment of cancer. Progress in structural studies [2] of these two physiologically and pathophysiologically important isoenzymes in complex with selective isoquinoline inhibitors will be discussed. Elucidation of inhibitor binding

to various isoforms can help in rational drug design of carbonic anhydrase inhibitors.

1. Sly and Hu *Annu. Rev. Biochem.* **64** (1995), pp. 375–401.
2. Gitto R., Agnello S., Ferro S., Luca L. De, Vullo D., Brynda J., Mader P., Supuran C.T., Chimirri A., *J. Med. Chem.*, in press.

L24**STRUCTURAL ANALYSIS OF PLANT AMINOALDEHYDE DEHYDROGENASES**

Martina Tylichová¹, David Kopečný¹, Pierre Briozzo², Solange Moréra³, Jacques Snégaroff² and Marek Šebela¹

¹*Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic.*

²*UMR206 Laboratoire de Chimie Biologique, INRA-AgroParisTech, F-78850 Thiverval-Grignon, France.*

³*Laboratoire d'Enzymologie et de Biochimie Structurales, CNRS, F-91198 Gif-sur-Yvette Cedex, France.*
Email: kopecny_david@yahoo.co.uk

Aminoaldehyde dehydrogenases (AMADH, EC 1.2.1.19) catalyze the terminal step in polyamine catabolism by oxidizing omega-aminoaldehydes like 4-aminobutyraldehyde (ABAL) and 3-aminopropionaldehyde (APAL). Based on their amino acid sequences, the enzymes belong to the same group as betaine aldehyde dehydrogenases (BADHs, EC 1.2.1.8). Presence of two isoenzymes is common in plants. To understand their function, we expressed two AMADHs of 503 amino acids from pea (*Pisum sativum*, PsAMADH1 and 2) and performed X-ray crystallographic study together with kinetic analysis using a large set of natural and synthetic aminoaldehydes. The structures of both PsAMADHs in complex with NAD⁺ coenzyme were refined at 2.4 Å and 2.15 Å resolution. They show that both plant enzymes are dimeric and provide a detailed description of the coenzyme and substrate binding site. Likewise,

we analyzed kinetic properties of two AMADHs from tomato (*Lycopersicon esculentum*, LeAMADH1 and 2) and two isoenzymes from maize (*Zea mays*, ZmAMADH1 and 2). Except for LeAMADH1, five remaining AMADHs carry C-terminal peroxisomal targeting sequence signal type 1 [S/A]KL and exhibit similar kinetic properties. Although these enzymes are often assigned to BADHs in different databases, our results show that this is no longer correct as all six studied enzymes preferred APAL and ABAL to betaine aldehyde as a substrate. Although substrate specificity can vary among species, in terms of wider substrate specificity the above-mentioned enzymes should be considered as AMADHs and not BADHs.

Supported by grant 522/08/0555 from the Czech Science Foundation and grant MSM 6198959215 from the Ministry of Education, Youth and Sports of the Czech Republic.

L25**DEFINING STRUCTURAL FEATURES OF THE FAMILY OF TETRAMERIC FLAVOPROTEINS WrbA**

J. Wolfová¹, J. Carey², J. Brynda^{1,3}, R. Ettrich^{1,4}, I. Kutá Smatanová^{1,4}

¹*Institute of Physical Biology, University of South Bohemia České Budějovice, Zámek 136, CZ-373 33 Nové Hradky, Czech Republic*

²*Chemistry Dep., Princeton University, Washington Rd and William St, Princeton, NJ 08544-1009, USA*

³*Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, CZ-16637 Prague 6, Czech Republic*

⁴*Institute of Systems Biology and Ecology, Academy of Science of the Czech Republic, Zámek 136, CZ-373 33 Nové Hradky, Czech Republic*

Flavoproteins WrbA form a distinct family of flavodoxin-like proteins that is widely distributed in living organisms, from bacteria to fungi and higher plants [1, 2]. These proteins were identified as tetrameric NADH:quinone oxidoreductases (E.C. 1.6.5.2) [2], carrying out two-electron transport from NADH to quinones by using flavin mononucleotide (FMN) as the redox-active cofactor

[2, 3, 4]. Together with other enzymes performing the two-electron reductions of quinones [5, 6, 7], members of the WrbA family are thought to participate in the cell protection against oxidative stress. Structural investigations of the prototypical WrbA from *Escherichia coli* and its homologues in other bacteria [8, 9] confirmed the previous observations and sequentially-based predictions that in each



structure four WrbA monomers form a tetramer, where individual subunits share the common fold of the related flavodoxins [10] with sequence insertions unique for WrbA family forming additional secondary structure elements. Unlike typically monomeric flavodoxins that are involved in one-electron transport processes between protein partners, tetrameric flavoproteins WrbA execute the two-electron reductions of quinones. To understand these functional distinctions and the unique tetramerization ability of WrbA in context with the molecular structure and to find the defining structural features of the WrbA family, the detailed comparative structural analysis of *E. coli* WrbA with the related flavodoxins and the functionally homologous eukaryotic FAD-dependent quinone oxidoreductase was performed.

Structural analysis included three structural models of *E. coli* WrbA obtained by our group using X-ray diffraction on single crystals [11]: two crystal structures of the protein complexed with FMN (holoWrbA, two crystal forms, PDB IDs: 2R96 and 2R97), one crystal structure of the protein without FMN bound (apoWrbA, PDB ID: 2RG1). The structures were compared by the 3D-superposition with long-chain holo- and apoflavodoxin from *Anabaena* (PDB IDs: 1FLV and 1FTG, respectively; [12, 13]) and with mammalian NAD(P)H:quinone oxidoreductase (Nqo, PDB ID: 1QRD; [7]).

Structural comparison of the monomers of holoWrbA and holoflavodoxin revealed only one characteristic sequence insertion distinguishing WrbA from flavodoxins, in contrast with previous reports. Structurally the unique insertions form small subdomains contacting each other at the 'poles' of the WrbA tetramers. Nevertheless, analysis of interfaces of the WrbA tetramers indicated that the key elements promoting tetramerization correspond to the integral secondary structure elements of the flavodoxin fold and thus the unique subdomain of WrbA is not dedicated to tetramerization as earlier proposed. Tetramer appears to be the obligate functional assembly of WrbA, with residues of the three subunits participating on the formation of the FMN-binding site. Comparison of the WrbA FMN-binding site with those of flavodoxin and Nqo showed that tetrameric WrbA forms a cavernous active site similar to that of dimeric Nqo, that analogously to WrbA promotes two-electron reduction of the electrophilic substrates. Assembly of the flavodoxin-like fold into tetramers to form active site of WrbA seems to be adaptation to the two-electron redox reaction. Despite the different FMN-binding

sites of WrbA and flavodoxin striking similarities were observed in the behavior of the FMN-binding residues in response to FMN binding.

The detailed comparative study of WrbA structures enabled specification of the defining structural features of the WrbA family and sharpening of the view of the relationship between WrbA, flavodoxins and eukaryotic quinone oxidoreductases. Surprising finding of unifying features with the related protein families indicates WrbA to be a significant member of flavodoxin-like proteins.

1. R. Grandori & J. Carey, *Protein Sci.*, **3** (1994) 2185-2193.
2. E.V. Patridge & J.G. Ferry, *J. Bacteriol.*, **188** (2006) 3498-3506.
3. R. Grandori, P. Khalifah, J.A. Boice, R. Fairman, K. Giovanelli & J. Carey, *J. Biol. Chem.*, **273** (1998) 20960-20966.
4. G. Nöll, E. Kozma, R. Grandori, J. Carey, T. Schödl, G. Hauska & J. Daub, *Langmuir*, **22** (2006) 2378-2383.
5. L. Ernster, *Chem. Scr.*, **27A** (1987) 1-13.
6. E. Cadenas, *Biochem. Pharmacol.*, **49** (1995) 127-140.
7. R. Li, M.A. Bianchet, P. Talalay & L.M. Amzel, *Prot. Natl. Acad. Sci.*, **92** (1995) 8846-8850.
8. J. Carey, J. Brynda, J. Wolfova, R. Grandori, T. Gustavsson, R. Ettrich & I. Kuta Smatanova, *Protein Sci.*, **16** (2006) 2301-2305.
9. J. Gorman & L. Shapiro, *Protein Sci.*, **14** (2005) 3004-3012.
10. R.P. Simonsen & G. Tollin, *Mol. Cell. Biochem.*, **33** (1980) 13-24.
11. J. Wolfova, I. Kuta Smatanova, J. Brynda, J. R. Mesters, M. Lapkouski, M. Kutý, A. Natalello, N. Chatterjee, S. Chern, E. Ebbel, A. Ricci, R. Grandori, R. Ettrich & J. Carey, *Biochim. Biophys. Acta*, **1794** (2009) 1288-1298.
12. S.T. Rao, F. Shaffie, C. Yu, K.A. Satyshur, B.J. Stockman, J.L. Markley & M. Sundarlingham, *Protein Sci.*, **1** (1992) 1413-1427.
13. C.G. Genzor, A. Perales-Alcon, J. Sancho & A. Romero, *Nat. Struct. Biol.*, **3** (1996) 329-332.

This work was supported by the Ministry of Education of the Czech Republic (Kontakt ME09016, MSM6007665808, LC06010, by the Academy of Sciences of the Czech Republic (AVOZ60870520) and by the U.S. National Science Foundation (INT03-09049 to J.C.).

SOLVING PHASE PROBLEM USING A SE-MET DERIVATIVE OF THE FLAVOENZYME NAD(P)H:ACCEPTOR OXIDOREDUCTASE (FERB)

T. Klumpler¹, J. Marek¹, V. Sedláček², I. Kučera²

¹Laboratory of Molecular Plant Physiology, Department of Functional Genomics and Proteomics, Inst. of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5/A2, CZ 625 00 Brno, Czech Rep.

²Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5/A5, CZ 625 00 BRNO, Czech Rep.
klumpler@sci.muni.cz

Keywords:

ferric reductase B, FerB, phasing, MRSAD, MAD

The flavin adenine dinucleotide-dependent enzyme FerB from *Paracoccus denitrificans* reduces a range of substrates, including chromate, ferric complexes, benzoquinones and naphthoquinones. The reduced form of nicotinamide adenine dinucleotide serves as a source of electrons. Recombinant unmodified and selenomethionine-substituted (Se-Met) FerB derivatives were crystallized, diffraction data for both forms were collected and the phase problem for Se-Met FerB dimer was solved by three-wavelength multiple anomalous dispersion, followed by the combination of molecular replacement and single-wavelength anomalous diffraction phasing. A molecular-replacement solution of unmodified FerB tetramer was obtained using Se-Met structure as a search model.

Introduction

The easiest way to crystal structure determination is the molecular replacement (MR). Only one native dataset, the coordinates of homologous structure and software that find the right number, orientation and translation of initial search model are needed. Two-third of more than 60 000 protein structures deposited in January 2010 in the Protein Data Bank [1] has been determined using MR techniques. Easy-to-use MR software is available and over 95% of deposited X-ray structures solved by MR has been determined using MR protocols of CNS [2], or one of specialized MR packages AMoRe [3], MOLREP [4] and PHASER [5]. More recently, software for automatic choice of phasing models from databases has been released, such as MrBUMP [6] and BALBES [7]. The solution of phase problem by MR could be very fast in principle, but often first model requires multiple rounds of manual refinement.

The second most important phasing techniques are based on intensity differences arising from the presence of heavy atoms implemented in single or multiple isomorphous replacement with or without anomalous scattering (SIR/MIR or SIRAS/MIRAS) or in single or multiple wavelength anomalous diffraction methods (SAD or MAD, [8]). MIR methods were used to determine the first X-ray structures of macromolecules and still have potential to determine unknown structure directly from experimental data. Heavy atoms are introduced to the protein crystal by soaking the crystal in the ionic solution of heavy atom. On the contrary, SAD and MAD methods incorporate heavy (typically selenium) atoms into protein crystals using protein molecules containing selenomethionine instead of

methionine [9]. Se-Met methods becomes more and more popular recently, because they eliminate problems associated with heavy-metal screening, the lack of isomorphism between native and heavy-atoms structures and mainly the only one single crystal is needed to perform complete diffraction experiment. Heavy-atom (or selenium) substructure could be determined using the Patterson or direct-methods programs such as SnB [10], SHELXD [11], CNS or SOLVE [12]. Determination of heavy-atom substructure via SAD can be initialized using preliminary positions of heavy-atom from an MR solution. This combined technique is called molecular replacement with single-wavelength anomalous diffraction (MRSAD) [13].

A broad scale of available programs indicates that crystallographer chooses the most appropriate individual programs for the specific sub-tasks executed during effective pass through the complete process of the protein crystal structure determination. At least partial automation of this multi-step decision process has become recent initiative. Different automated pipeline have been built up, e.g. ACrS [14], Auto-Rickshaw [15], autoSHARP [16], CRANK [17], ELVES [18], HKL-3000 [19], PHENIX [20], SGXPro [21].

The flavin-dependent enzyme FerB from *Paracoccus denitrificans* reduces a broad range of compounds, including ferric complexes, chromate and quinones, at the expense of the reduced nicotinamide adenine dinucleotide cofactors, NADH or NADPH [22, 23]. Enzymes utilizing flavin cofactors, (flavin mononucleotide, FMN, or flavin adenine dinucleotide, FAD), are unique in their ability to catalyze a wide variety of mechanistically different reactions, such as dehydrogenation, oxygen activation, halogenation, non-redox conversions, light sensing and emission, and DNA repair [24]. The function of all of these enzymes in cell metabolism has not yet been fully elucidated. Finding the molecular basis of the catalysis by FerB would be greatly aided by knowledge of the three-dimensional structure of the enzyme. Here we report the solution of the phase problem of the Se-Met derivative of flavin dependent enzyme FerB from *Paracoccus denitrificans* using the advanced 3W-MAD and MRSAD protocols of Auto-Rickshaw: the EMBL-Hamburg automated crystal structure determination platform after MR solution of the native protein FerB had failed.



Materials and methods

1. Production, purification and crystallization of Se-Met FerB

Se-Met FerB was prepared using the methionine biosynthesis inhibition method [25]. Purification in a single chromatography step using a HisPrep FF 16/10 column (GE Healthcare) [26] resulted in almost homogenous protein preparations (> 95% homogeneity). Purity and monodispersity of a sample were controlled by SDS-PAGE electrophoresis and dynamic light scattering. For protein crystallization micro-seeding technique was exploited and crystals of native and Se-Met FerB were obtained (Fig. 1.). For details see [27].

2. MALDI-TOF mass spectrometry

Native and Se-Met FerB were analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a ULTRAFLEX III mass spectrometer (Bruker Daltonics, Germany). Samples were co-crystallized with 2,5-dihydroxybenzoic acid and analyzed in linear mode using an accelerating voltage of 25 kV. The instrument was calibrated with $[\text{MH}]^+$ and $[\text{MH}]^{2+}$ peaks using a mixture of peptide standards (Bruker Daltonics). Peaks at molecular masses of 21289 and 21616 detected for intact and Se-Met FerB correspond well to the predicted mass difference of 328 Da (seven methionine residues per chain).

3. Auto-Rickshaw structure determination

Se-Met diffraction data were collected at tunable beamline X12 of the DORIS-III storage ring at EMBL/DESY (Hamburg, Germany), processed and merged using the XDS system [28] (for details see [27]). The structure of Se-Met FerB was solved using the advanced 3W-MAD and MRSAD protocols of Auto-Rickshaw: the EMBL-Hamburg automated crystal structure determination platform. The output diffraction data from XDS were converted for use in Auto-Rickshaw using programs of the CCP4 suite (CCP4, 1994), and F_A values were calculated using the program SHELXC [11]. Based on an initial analysis of the data, the maximum resolution for FerB substructure determination and initial phase calculation was set to 1.8 Å. 14 selenium atoms were found using the program SHELXD. The correct hand for the substructure was determined using the programs ABS [29] and SHELXE [11] and initial phases were calculated after density modification using the program SHELXE. The initial phases were improved by density modification and phase extension using the program DM [30]. Then the twofold non-crystallographic symmetry (NCS) operator was found and then density modification with solvent flattening and NCS averaging was applied, both using the program RESOLVE [31]. Resulting phases from RESOLVE were used as input for model building mode of program ARP/wARP.

The model building and refinement protocol implemented into MRSAD pipeline of Auto-Rickshaw started with rigid-body refinement of individual protein chains at 4 Å resolution followed by positional, B-factor and once

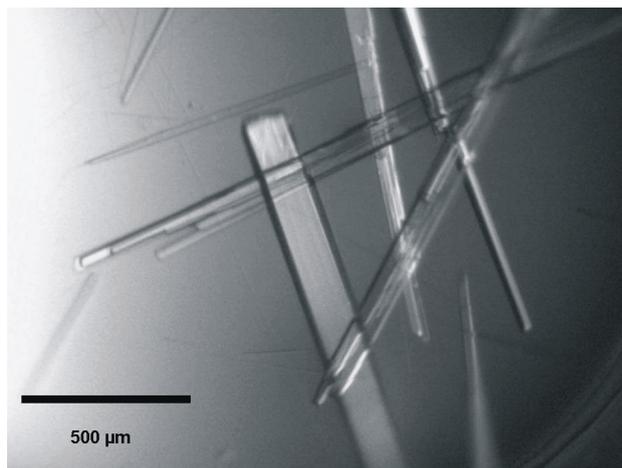


Figure 1. Rod-like crystals of Se-Met FerB.

more positional refinement at 3.0 Å using CNS. The CNS result was used for refinement and phase extension to 1.75 Å resolution using REFMAC5 [32]. In the next step of the protocol, quality of electron density map was improved using density modification and NCS-averaging by RESOLVE (Fig. 2.). New, more complete model of FerB was prepared consequently building of polyalanine model by beta version of SHELXE, side-chain docking with RESOLVE, REFMAC5 refinement, and finally by run of ARP/wARP [33, 34] in the model building regime.

Results

We had tried to solve FerB structure by the molecular replacement (MR) methods using an NAD(P)H dependent FMN reductase flavoprotein from *Pseudomonas aeruginosa* PA01 (PDB code 1RTT) [35] identified by a FASTA search [36] as a model. Unfortunately, all MR trials were unsuccessful. We therefore collected data with selenomethionine derivative of FerB. An excellent data quality and their maximum resolution allow us to try to solve phase problem of FerB using the advanced 3W-MAD protocol of Auto-Rickshaw: the EMBL-Hamburg automated crystal structure determination platform. The twofold NCS operator connecting expected two FerB monomers close to $x, -y, ?-z$ was found by RESOLVE. The model building mode of program ARP/wARP employed at the end of the advanced 3W-MAD protocol of Auto-Rickshaw was able to find 309 (from expected 364) residues divided to 11 chains, 100% of them have been docked in FerB sequence, and this intermediate model was completed by the MRSAD pipeline of Auto-Rickshaw to almost complete model of Se-Met FerB homodimer containing 349 residues divided into 9 chains (340 of them correctly docked) with final $R/R_{free} = 0.2144/0.2682$.

The structure of native FerB tetramer was successfully solved by molecular replacement technique implemented in PHASER (the final translation function Z-score TFZ = 66.6) with manually unrefined structure of one of Se-Met FerB monomers as the search model. Refinement of both FerB structures is now in progress.

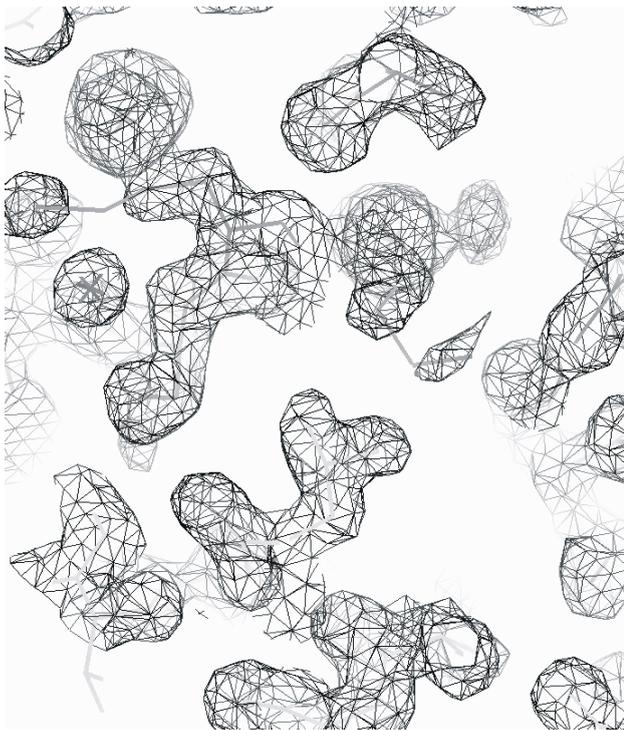


Figure 2. $2F_o - F_c$ map of Se-Met FerB contoured at 1σ .

We wish thank to the EMBL/DESY Hamburg for providing us with synchrotron facilities and D. Tucker for his assistance with data collection on beamlines X12 and X13 of the DORIS-III storage ring at DESY Hamburg. The authors are grateful to Ondrej Šedo for measuring the MALDI-TOF MS spectra and the Meta Center for computer time. This research was supported by grants from the Czech Science Foundation (grant Nos. 204/08/H054 and 525/07/1069 and P503/10/217) and the Ministry of Education, Youth and Sports (grant Nos. MSM0021622413, MSM0021622415 and LC06034).

References:

- 1 H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne, *Nucleic Acids Res.*, **28** (2000), 235.
- 2 A.T. Brünger, P.D. Adams, G.M. Clore, W.L. DeLano, P. Gros, R.W. Grosse-Kunstleve, J.S. Jiang, J. Kuszewski, M. Nilges, N.S. Pannu, R.J. Read, L.M. Rice, T. Simonson, G.L. Warren, *Acta Cryst.*, **D54**, (1998), 905.
- 3 J. Navaza, *Acta Cryst.*, **A50**, (1994), 157.4 A. Vagin, A. Teplyakov, *J. Appl. Cryst.*, **30**, (1997), 1022.
- 5 A.J. McCoy, R.W. Grosse-Kunstleve, P.D. Adams, M.D. Winn, L.C. Storoni, R.J. Read, *J. Appl. Cryst.*, **40**, (2007), 658.
- 6 R.M. Keegan, M.D. Winn, *Acta Cryst.*, **D63**, (2007), 447.
- 7 F. Long, A.A. Vagin, P. Young, G.N. Murshudov, *Acta Cryst.*, **D64**, (2008), 125.
- 8 W.A. Hendrickson, *Science*, **254**, (1991), 51.
- 9 W.A. Hendrickson, J.R. Horton, D.M. LeMaster, *EMBO J.*, **9**, (1990), 1665.
- 10 R. Miller, N. Shah, M.L. Green, W. Furey, C.M. Weeks, *J. Appl. Cryst.*, **40**, (2007), 938.
- 11 G.M. Sheldrick, *Acta Cryst.*, **A64**, (2008), 112.
- 12 T.C. Terwilliger, J. Berendzen, *Acta Cryst.*, **D55**, (1999), 849.
- 13 J.P. Schuermann, J.J. Tanner, *Acta Cryst.*, **D59**, (2003), 1731.
- 14 J.S. Brunzelle, P. Shafae, X. Yang, S. Weigand, Z. Ren, W.F. Anderson, *Acta Cryst.*, **D59**, (2003), 1138.
- 15 S. Panjikar, V. Parthasarathy, V.S. Lamzin, M.S. Weiss, P.A. Tucker, *Acta Cryst.*, **D65**, (2009), 1089.
- 16 C. Vonrhein, E. Blanc, P. Roversi, G. Bricogne, *Methods Mol. Biol.*, **364**, (2006), 215.
- 17 S.R. Ness, R.A. de Graaff, J.P. Abrahams, N.S. Pannu, *Structure*, **12** (2004), 1753.
- 18 J. Holton, T. Alber, *Proc. Natl Acad. Sci. USA*, **101**, (2004), 1537.
- 19 W. Minor, M. Cymborowski, Z. Otwinowski, M. Chruszcz, *Acta Cryst.*, **D62**, (2006), 859.
- 20 P.D. Adams, R.W. Grosse-Kunstleve, L.W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter, & T.C. Terwilliger, *Acta Cryst.*, **D58**, (2002), 1948.
- 21 Z.Q. Fu, J. Rose, B.C. Wang, *Acta Cryst.*, **D61**, (2005), 951.
- 22 J. Mazoch, R. Tesařík, V. Sedláček, I. Kučera, J. Turánek, *Eur. J. Biochem.*, **271**, (2004), 553.
- 23 V. Sedláček, R.J.M. van Spanning, I. Kučera, *Arch. Biochem. Biophys.*, **483**, (2009), 29.
- 24 A. Mattevi, *Trends Biochem. Sci.*, **31**, (2006), 276.
- 25 G.D. Van Duyne, R. Standaert, P.A. Karplus, S.L. Schreiber, J. Clardy, *J. Mol. Biol.*, **229**, (1993), 105.
- 26 R. Tesařík, V. Sedláček, J. Plocková, M. Wimmerová, J. Turánek, I. Kučera, *Protein Express. Purif.*, **68**, (2009), 233.
- 27 T. Klumpler, V. Sedláček, J. Marek, M. Wimmerová, I. Kučera, *Acta Cryst.*, **F66**, (2010), (in press).
- 28 W. Kabsch, *J. Appl. Cryst.*, **26**, (1993), 795.
- 29 Q. Hao, *J. Appl. Cryst.*, **37**, (2004), 498.
- 30 K. Cowtan, *Joint CCP4 and ESF-EACBM Newsletter on protein crystallography*, **31**, (1994), 34.
- 31 T.C. Terwilliger, *Acta Cryst.*, **D59**, (2003), 38.
- 32 G.N. Murshudov, A.A. Vagin, E.J. Dodson, *Acta Cryst.*, **D53**, (1997), 240.
- 33 A. Perrakis, R.J. Morris, V.S. Lamzin, *Nature Struct. Biol.*, **6**, (1999), 458.
- 34 S.X. Cohen, M. Ben Jelloul, F. Long, A. Vagin, P. Knipscheer, J. Lebbink, T.K. Sixma, V.S. Lamzin, G. N. Murshudov, A. Perrakis, *Acta Cryst.*, **D64**, (2008), 49.
- 35 R. Agarwal, J.B. Bonanno, S.K. Burley, S. Swaminathan, *Acta Cryst.*, **D62**, (2006), 383.
- 36 W.R. Pearson, D.J. Lipman, *Proc. Natl. Acad. Sci. USA*, **85**, (1998), 2444.



Lectures - Friday, March 19, afternoon, Session VI

L27

BIOINFORMATIC ANALYSES OF THE FIRST SOLVATION SHELL OF NUCLEIC ACIDS

Bohdan Schneider

*Institute of Biotechnology AS CR, v.v.i., Vídeňská 1083, CZ-142 20 Prague, Czech Republic;
http://www.structbio.eu/BS/*

Relative humidity, quality and quantity of cations and other solvent species and solvation in general determine thermodynamic and structural behavior of nucleic acids. Structures determined by X-ray crystallography provide a unique information about the first solvation shells of biomolecules. We studied various aspects of the first solvation shell of DNA and RNA by analysis of distributions of water and metal cations around nucleotides in publically deposited crystal structures of nucleic acids. Detailed analysis of hydration in double helical DNA showed that their first hydration shell is well localized, stereochemistry of the preferred positions of water molecules around bases and phosphates, so called hydration sites, is determined locally, i.e. primarily by geometry of the hydrogen bond, but

the exact location of the hydration sites adjusts to the nucleic acid conformation and sequence. This is in general true also for metal binding sites around phosphates but variability of their positions is lower than that of water positions and especially the magnesium binding site to the phosphate group is almost unchanged in DNA or RNA of various conformations. Hydration sites can serve as markers for optimal binding positions of hydrophilic drug and protein functional groups, and perhaps surprisingly, also to their hydrophobic groups.

The reviewed solvation projects have been mostly supported by NSF grants to the Nucleic Acid Database, NDB. The support of the Institute of Biotechnology AS CR, v.v.i. (AV0Z50520701) is acknowledged.

L28

USING THE CAMBRIDGE STRUCTURE DATABASE OF ORGANIC AND ORGANOMETALIC COMPOUNDS IN STRUCTURE BIOLOGY

Jindřich Hašek

*Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic,
Heyrovského nám. 2, Praha 6, Czech Republic
hasek@imc.cas.cz*

Introduction

Experimentally determined structures are collected in several separate databases. Macromolecular structures of biological interest are collected in the Protein Data Bank (PDB) /5/, polymer structures in PolyBase /6/, inorganic structures in Database of Inorganic Crystal Structures (http://www.fiz-karlsruhe.de/icsd_content.html), structures of metals and alloys in CRYSMET@ database (<http://www.tothcanada.com/databases.htm>). Organic structures of biological interest discussed in this paper are collected in the Cambridge Structure Database (CSD).

Cambridge Structure Database

The CSD maintained by the Cambridge Crystallographic Data Center (CCDC) contains more than half million of organic and organometallic compounds [1,2]. Most structures deposited in the database are determined experimentally by diffraction methods with high accuracy. Coordinates of atoms have standard deviations usually in the range 0.001 – 0.01 Å (i.e. better than 1 picometer). With this accuracy, the CSD is useful source of information on bond strengths,

ionization states, etc. It provides also a reliable information on electron density of hydrogens that is usually not available in the PDB (Fig.1).

About hundred thousand compounds deposited in the CSD are of biological relevance. One can find here many drug leads, peptides, biologically active compounds, etc. About six thousands compounds of biological origin are labeled as natural products.

The CSD is used at more than a thousand universities from 69 countries and at about 200 commercial organizations from 15 countries. Most installations are in the USA (243), Japan (105), France (99), Germany (69), Russia (59), Poland (55), Spain (51), Italy (40), GB (37), Canada (35). The Czech Republic belongs to the first five states with permanent subscription to the CSD since 1972. Non-commercial users from the Czech Republic can use the CSD via registration at the Institute of Physics of the Academy of Sciences in Praha (contact: dusek@fzu.cz) or can install their local versions at their own personal computers (contact: hasek@imc.cas.cz). A basic information

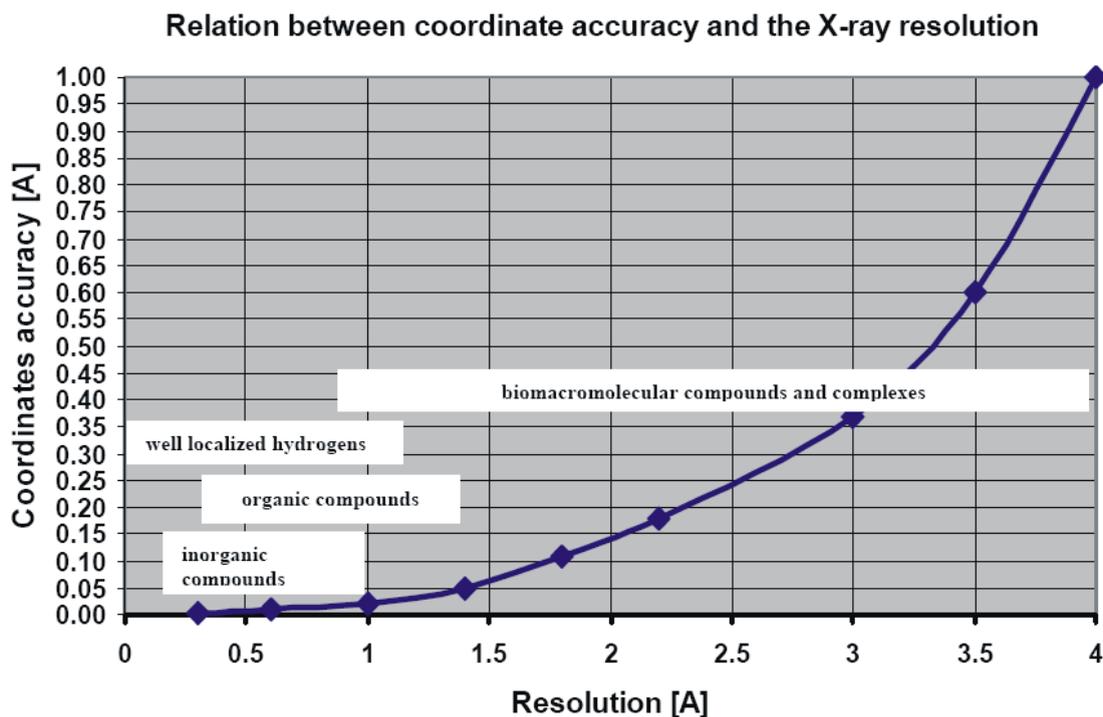


Figure 1. Relation between the diffraction limit (resolution) and the estimated standard deviation of atomic positions for structures determined by diffraction methods (1 Å = 100 pm).

on the database [3,4], on the related software, and on free services is available at the address

<http://www.ccdc.cam.ac.uk/products/>.

Applications

Searching for structural properties of groups of the compounds extracted from CSD and using statistical tools one should remember that the contents of database does not cover the space of compounds uniformly, i.e. the extracted set of structures is not a statistically random sample. For example in peptide analysis, the conformationally interesting aminoacid residues (AA) are represented much more frequently in CSD than other residues. There are several hundreds of peptides with prolin or glycin in CSD because an interest of people has been concentrated especially to the structural effects of different sequences containing these two aminoacids. Other AAs are found much less frequently.

Another example is a limited crystallizability of long peptides. Short peptides have usually well defined conformations and can be crystallized easily. Longer peptides with 10–25 AA are already fully hydrated (Fig.2), but still they are not long enough to form a stable 3D structure in solution. As a result, they are conformationally unstable, their crystallization is very difficult and therefore one can find only a few structures in the CSD.

CCDC provides also software for analysis of the Protein Data Bank (PDB) of experimentally determined bio-macromolecular structures [5]. The efficiency and accuracy of structure determination by X-ray crystallography

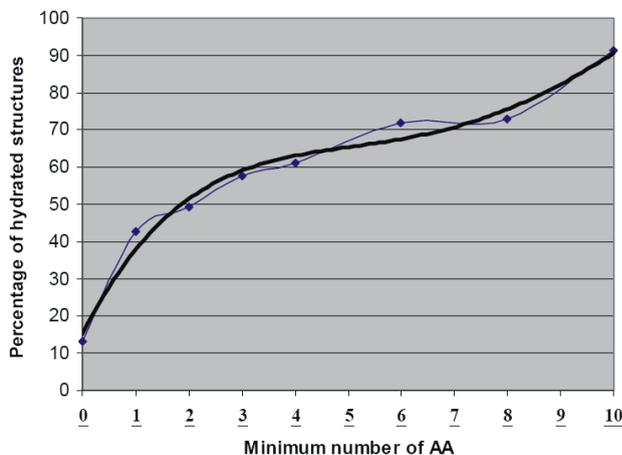


Figure 2. Probability of hydrated crystals increases with the number of aminoacid residues.

depends mainly on the diffraction quality of crystals (Fig.1) and in principle has no limitation as for the complexity of the macromolecular complexes. Thus, a search for 3D fragments and analysis of these large macromolecular complexes with millions of atoms is demanding. Software “Relibase” provided by CCDC is very useful for 3D scanning of the PDB contents and the program “GOLD” is designed for empirically based 3D ligand docking interesting for special interest groups of structure biologists and drug designers. The synthetic polymers col-



lected in the Polymer Structure Database (PolyBase) [6] can be analyzed by CCDC program MERCURY.

Conclusion

The CSD is available for non-commercial users in the Czech Republic for a small license fee paid each year. The related software for extension of structure analysis based on the CSD and PDB data can be purchased separately. A free evaluation copy can be requested for any CCDC product at the address http://www.ccdc.cam.ac.uk/support/product_references/. For teaching purposes, one should register via internet for a free version of CSD containing 500 structures http://www.ccdc.cam.ac.uk/free_services/teaching/.

The activities reported in this paper are supported partly from the GA AS project IAA500500701 and the GA CR project 305/07/1073.

L29

METACENTRUM - ON THE WAY FROM DISTRIBUTED COMPUTING TO CYBER INFRASTRUCTURE FOR RESEARCH

J. Kmuníček¹, V. Žáková², S. Licehammer², M. Kuba¹ and I. Křenková¹

¹CESNET, z.s.p.o., Žitkova 4, 160 00 Praha 6, Czech Republic

²Faculty of Informatics, Masaryk University, Botanická 68a, 620 00 Brno, Czech Republic
kmunicek@ics.muni.cz

Nowadays, the research in any scientific field became truly global and internationalized. The current advances in development, deployment and subsequent support of distributed collaborative environments created a uniform, persistent, pervasive and scalable digital worldwide infrastructure (1). The Czech Republic participates within these activities through the national e-Infrastructure project MetaCentrum (2). MetaCentrum - a CESNET activity - is responsible for building the National Grid and its integration (3) to the related international activities. MetaCentrum operates and manages distributed computing infrastructure consisting of computing and storage resources owned by CESNET as well as resources of co-operative academic centres within the Czech Republic. Through supporting advanced services to all scientific disciplines, MetaCentrum provides added values for end users from many interdisciplinary areas as life science, biomedicine and many others, including naturally structural biologists.

1. Allen F.H., Motherwell W.D.S. Acta Crystallogr. B 58, 407-422, (2002).
2. Taylor R., Acta Crystallogr. D58, 879-888, (2002).
3. CCSD manual "Using the Cambridge Structural Database for Teaching", 103 pp., CCDC Cambridge (2008).
4. CCSD manual "Conquest 1.7 User Guide", 226 pp., CCDC Cambridge (2004).
5. Berman H. M., Westbrook J., Feng Z., Gilliland G., Bhat T. N., Weissig H., Shindyalov I. N., Bourne, P. E. Nucleic Acids Res. 28, 235-242, (2000).
6. Hašek J., Labský J.: Database of polymer structures - PolyBase, CSCA Praha (1995).

MetaCentrum matured services in the field of virtualisation, security, job scheduling and the utilisation of advanced network features provided by the CESNET2 network are ready to serve for shaping up the new era of structural biology. If you are looking how structural biology domain can substantially benefit from MetaCentrum and worldwide provided production services consider following questions: Would you like to boost your research using the collaboration platform and available tools easing your international research cooperation? Are you hungry for more compute power to speed up your research? Are you looking how to scale up your (bio)molecular systems of interest you currently investigate at atomic details? Do you need to store, access and handle huge amounts of data or archive your research results? You have your specific requests. MetaCentrum has more than corresponding solutions to offer.



L30

THREE DIMENSIONAL INFORMATION STRUCTURE OF LIVING CELL FATE

D. Štys, J. Urban, T. Náhlík, J. Vaněk, T. Levitner, P. Císař

*Institute of Physical Biology, University of South Bohemia, Zámek 136, 373 33 Nové Hradky, Czech Republic
stys@jcu.cz*

Living cell is best examined by time-resolved optical microscopy. The holy grail of the approach is fast capture of inasmuch complete three-dimensional information about the cell with spatial resolution sufficient to capture all relevant objects, spectral resolution which enables to characterize their chemical composition and time resolution sufficient to capture all relevant events. In the previous sentence there are numerous vaguely defined terms which may only be defined only with respect to the appropriate model of the cell structure (equivalent of the state space in mechanics) and cell dynamics (moment components of the phase space).

In ideal case we should be able to follow the trajectory of the cell in the multidimensional chemico-mechanical phase space in continuous time. In any real case, we have only real capture of a slice of a mechanical space at a time instant and have no realistic control over the identity of element of the chemical space – equivalent of component in thermodynamic terminology. Cells are dynamic systems exhibiting asymptotic stability. Such behaviour is expected by non-linear dynamic systems where in the state space on obtains regions of asymptotic stability which are populated with significantly higher probability than rest of the state space [1]. These are the objects which we observe at our given timescale.

This natural premise, plus generalised stochastic systems theory [2] which brings the observation into the reality of the measurement, forms the theoretical basis and the framework for analysis of the observation, the elements of the model of cell monolayer. In relation to them we may analyse the information content of the measurement [3]. Thus, we expect to observe individual dynamic objects characterised by structural similarity, characteristic color-

ation (i.e. colours, their heterogeneity, dynamics) and oscillations between several observable states.

The information channel – the microscope – is characterised by the point-spread function, a recipe by which a microscopic object is depicted at the destination – camera screen. The point spread function may be completely understood only for objects of known structure sufficiently separated from each other. This condition is never satisfied in living cell microscopy. The microscopy image, nevertheless, carries nearly complete information about the multidimensional mechanico-chemical state space and may be extended to approximation of the phase space.

In this paper we report (I) mathematical description of the observable state space in cell microscopy, (II) partial description of the information content of the optical microscopy measurement, (III) observation of certain elements of the model of cell monolayer.

1. P. Cvitanovi'c, R. Artuso, R. Mainieri, G. Tanner and G. Vattay, *Chaos: Classical and Quantum* (Niels Bohr Institute, Copenhagen 2009); ChaosBook.org/version13.
2. Žampa, P. and Arnošt, R. (2004) Alternative approach to continuous-time stochastic systems definition. In *Proceedings of the 4th WSEAS Conference*. Wisconsin.
3. Štys D., Urban J., Vaněk J. and Císař P., Analysis of biological time-lapse microscopic experiment from the point of view of the information theory, *Micron* 2010 in press

This work was partly supported by the Ministry of Education, Youth and Sports of the Czech Republic under the grant MSM 6007665808 and grant HCTFOOD A/CZ0046/1/0008 of EEA funds.