



L7

## PROTEIN CRYSTALLOGRAPHY AT THE UNIVERSITY OF SOUTH BOHEMIA

Ivana Kutá Smatanová

*Institute of Physical Biology University of South Bohemia and Institute of Systems Biology and Ecology AS CR, Zamek 1336, 373 33 Nove Hradky, ivanaks@seznam.cz*

The Laboratory of Crystallogenes and Biomolecular Crystallography (LCBC, formerly named Laboratory of Crystallogenes) was established in 2002 as a part of the Institute of Physical Biology of the University of South Bohemia. Later in 2005 the LCBC was renamed and began a part of Institute of Systems Biology and Ecology Academy of Sciences of the Czech Republic as well. The laboratory is located at the Academic and university center in Nove Hradky.

The aims of the LCBC are crystallization and structural studies of membrane and soluble biological macromolecular complexes using methods of X-ray diffraction as well as development of new alternative crystallization techniques and their testing.

X-ray crystallography is the major technique to get the structure of biological macromolecules at atomic resolution. These protein structures are central to understand the detailed mechanisms of biological processes and to discover novel therapeutics using a structure-based approach. Almost weekly, known scientific journals as Science, Nature or Cell feature striking drawings of large biological molecular assemblies and appropriate articles describe the role and function in their biological environments. To counter the myriad existent threats of diseases and terrorism, there is an urgent need to interpret the relevance of macromolecular structures published nowadays. We have crystallized several for living on the earth important soluble proteins (e.g. flavodoxin-like protein WrbA of *E.coli* [2, 6-7, 10, 12, 17], cytochrome of photosynthetic bacteria *Thiocapsa roseopersicina* [9, 13, 16], HsdR subunit of the type IB restriction enzyme EcoR124I [3, 11] DhaA04, DhaA12, DhaA14, DhaA15 [1, 8] and DhaAwt from *Rhodococcus rhodochromus* NCIMB 13064 in free form and in complex with isopropanol, DhaAwt in complex with trichloropropane, DhaA13 in complex with trichloropropane, DhaA31 in free form and in complex with trichloropropane, DhaA13 in complex with coumarine, DbeA from *Bradyrhizobium elkanii* USDA94 [4], DbeA1, Spur from *Strongylocentrotus purpuratus*) and also membrane protein complex (monomeric and dimeric core complex of photosystem II from higher plant *Pisum sativum*) [5, 14-15]. These protein complexes have been crystallized using standard, advanced and alternative crystallization techniques. Obtained monocrystals have been measured at the synchrotron radiation sources and diffraction data have been used to solve protein structures.

The LCBC is fully equipped for protein crystallization experiments and internationally recognized due to a biennial FEBS Advanced Course "Advanced methods in macromolecular crystallization". Analytical balance, IWA distiller for redistilled water, deep-freeze, iceboxes, incubators, ice generator, pH-meters, BioRad set for electro-

phoresis, high-magnification stereomicroscopes, Eppendorf pipette sets, commercial crystallization kits, chemicals, crystallization plates and all necessary plastic and glass crystallization items of Hampton Research (CA, USA), Molecular Dimensions Ltd (UK), JenaBioScience GmbH (Germany) or Triana SciTech (Spain) are available for direct use.

The LCBC actively cooperates with scientists from other lab in Czech Republic and from abroad. Long-term cooperation with the group of Prof. Juan Manuel Garcia-Ruiz (LEC Granada, Spain) and group of Prof. Rolf Hilgenfeld (University of Luebeck, Germany) has yielded important results on using of advanced counter-diffusion technique to crystallize membrane protein complex as well as implementation of macromolecular crystallography to teaching activities and establishment of new training course at the University of South Bohemia named FEBS practical and lecture course "Advanced Methods in Macromolecular Crystallization" that is biennially organized under auspices of Federation of European Biochemical Societies ([www.img.cas.cz/igm/cc](http://www.img.cas.cz/igm/cc)) and was marked as one of the best teaching courses in the Czech Republic.

Since 2002 16 students have worked on different crystallization projects in the frame of their graduate and postgraduate studies. 5 of them successfully finished their postgraduate studies and now they work in well-known laboratories in USA and Europe.

Ivana Kuta Smatanova is a head of the LCBC and since 2000 is responsible for 15 own national and international scientific projects\* aimed at protein crystallization and crystallography.

\* *ME CR: LC06010, ME09016, CZ.04.1.03/3.2.15.1/0094, ME640*

*GA CR: 206/03/D061, 206/00/D007*

*EU: FEBS PLC10-015, FEBS PLC08-002, FEBS PLC06-35, FEBS AC04-13*

*AIP CR: KONTAKT 6-06-14*

*AV CR a C.S.I.C.Spain: 2001CZ0001, 2004CZ0003, 2006CZ0011*

*IMB Jena Germany: HPRI-1999-00038.*

### Acknowledgement

*ME CR (LC06010, Kontakt ME09016, MSM6007665808) and AS CR (AV0Z60870520) support this work.*



1. Martin Klvana; Martina Pavlova; Tana Koudelakova; Radka Chaloupkova; Pavel Dvorak; Alena Stsiapanava; Michal Kutý; Ivana Kuta-Smatanova; Jan Dohnalek; Petr Kulhanek; Rebecca C. Wade; Jiri Damborsky: Pathways and Mechanisms for Product Release in the Engineered Haloalkane Dehalogenases Explored using Classical and Random Acceleration Molecular Dynamics Simulations. *J. Mol. Biol.* 392, 1339-1356 (2009), doi:10.1016/j.jmb.2009.06.076.
2. Julie Wolfova, Ivana Kuta Smatanova, Jiri Brynda, Jeroen R. Mesters, Mikalai Lapkouski, Michal Kutý, Antonino Natalello, Neal Chatterjee, Sy-Yeu Chern, Erin Ebbel, Angela Ricci, Rita Grandori, Rüdiger Ettrich, and Jannette Carey: Structural organization of WrbA in apo- and holo-protein crystals. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1794, 1288-1298 (2009), doi: 10.1016/j.bbapap.2009.08.001.
3. Mikalai Lapkouski, Santosh Panjikar, Pavel Janscak, Ivana Kuta Smatanova, Jannette Carey, Rudiger Ettrich, Eva Csefalvay: Structure of the motor subunit and translocation model for EcoR124I restriction-modification complex. *Nature Structural & Molecular Biology* 16(1), 94-5 (2009), doi: 10.1038/nsmb.1523.
4. Tatyana Prudnikova, Tomas Mozga, Pavlina Rezacova, Radka Chaloupkova, Yukari Sato, Yuji Nagata, Jiri Brynda, Michal Kutý, Jiri Damborsky, and Ivana Kuta Smatanova: Crystallisation and preliminary X-ray analysis of a novel haloalkane dehalogenase DbeA from *Bradyrhizobium elkani* USDA94. *Acta Cryst.* F65, 353-356 (2009).
5. J. Kohoutová, I. Kutá Smatanová, J. Brynda, M. Lapkouski, J. L. Revuelta, J. B. Arellano, R. Ettrich: Crystallization and preliminary crystallographic characterization of the extrinsic PsbP protein of photosystem II from *Spinacia oleracea*. *Acta Cryst.* F65, 111-115 (2009).
6. J. Wolfová, J. Brynda, J. R. Mesters, R. Ettrich, J. Carey, I. Kutá Smatanová: Structural changes of tetrameric flavoprotein WrbA upon flavin binding. *Materials Structure* 16, 2a, k56-57 (2009).
7. Julie Wolfová, Jiří Brynda, Jeroen R. Mesters, Jannette Carey, Rita Grandori and Ivana Kutá Smatanová: Crystallographic study of *Escherichia coli* flavoprotein WrbA, a new NAD(P)H-dependent guanine oxidoreductase. *Materials Structure* 15, 1, 55-57 (2008).
8. Alena Stsiapanava, Tana Koudelakova, Mikalai Lapkouski, Martina Pavlova, Jiri Damborsky and Ivana Kuta Smatanova: Crystals of DhaA mutants from *Rhodococcus rhodochrous* NCIMB 13064 diffracted to ultra high resolution: crystallization and preliminary diffraction analysis. *Acta Cryst.* F64, 137-140 (2008).
9. Ivana Tomčová and Ivana Kutá Smatanová: Copper co-crystallization and divalent metal salts cross-influence effect – a new optimisation tool improving crystal morphology and diffraction quality. *Journal of Crystal Growth* 306, 383-389 (2007).
10. Julie Wolfová, Jeroen R. Mesters, Jiří Brynda, Rita Grandori, Antonino Natalello, Jannette Carey and Ivana Kutá Smatanová: Crystallization and preliminary diffraction analysis of *E. coli* WrbA in complex with its cofactor flavin mononucleotide. *Acta Cryst.* F63, 571-575 (2007).
11. M. Lapkouski, S. Panjikar, I. Kuta Smatanova and E. Csefalvay: Purification, crystallization and preliminary X-ray analysis of the HsdR subunit of the EcoR124I endonuclease from *E. coli*. *Acta Cryst.* F63, 582-585 (2007).
12. Jannette Carey, Jiří Brynda, Julie Wolfová, Rita Grandori, Tobias Gustavsson, Rudiger H. Ettrich and Ivana Kutá Smatanová: WrbA bridges bacterial flavodoxins and eukaryotic NAD(P)H:quinone oxidoreductases. *Protein Science* 16, 10, 2301-2305 (2007).
13. Ivana Tomčová and Ivana Kutá Smatanová: Cross-crystallization as a new optimization tool of crystallization procedures. *Materials Structure* 14, 1, 3-5 (2007).
14. Tatyana Prudnikova, Michal Kutý, José A. Gavira, Peter Palenčár, František Vácha, Pavlína Řezáčová, Juan M. Garcia-Ruiz and Ivana Kutá Smatanová: Crystallization and structure-functional study of the photosystem II from higher plants. *Materials Structure* 14, 1, 5-7 (2007).
15. Ivana Kutá Smatanová, José A. Gavira, Pavlína Řezáčová, František Vácha, and Juan M. Garcia-Ruiz: New techniques for membrane protein crystallization tested on photosystem II core complex of *Pisum sativum*. *Photosynthesis Research* 90 (3), 255-259 (2006).
16. Ivana Tomčová, Rui Miguel Mamede Branca, Gabriella Bodó, Csaba Bagyinka, and Ivana Kutá Smatanová: Cross-crystallization and preliminary diffraction analysis of a novel di-heme cytochrome c4. *Acta Cryst.* F62, 820-824 (2006).
17. Julie Wolfova, Rita Grandori, Erika Kozma, Neal Chatterjee, Jannette Carey and Ivana Kuta Smatanova: Crystallization of the flavoprotein WrbA optimized by using additives and gels. *Journal of Crystal Growth* 284, 3-4, 502-505 (2005).