

Progress Report for 2007

(4.1.1. POPIS ŘEŠENÍ PROJEKTU)

IDENTIFICATION NUMBER	LC06063 (2006-2010)
TITLE OF THE PROJECT	Fluorescence microscopy in biological and medical research
COORDINATOR	Hof Martin Doc. Dr. rer.nat., DSc.
PARTICIPANTS	Kubínová Lucie RNDr. CSc. Hozák Pavel Prof. RNDr. DrSc. Palková Zdena Doc.RNDr. CSc. (Dr. Blahoš is due to his move (1.1.2007) to the Institute of Molecular Genetics member of Prof. Hozák's team)
NUMBER OF APPEARED AND ACCEPTED FULL PUBLICATIONS WITHIN FIRST 22 MONTHS	26 (average impact factor 3.3)
OBTAINED ACADEMIC DEGREES WITHIN FIRST 22 MONTHS	12 (3 MGR, 6 PHD, 2 DSC., 1 PROF.)

A. Overview on Scientific Activities

Fluorescence illumination and observation is the most rapidly expanding microscopy technique employed today, both in the medical and biological sciences, a fact which has spurred advances in chromophore and fluorophore technology as well as the development of a series of new fascinating technical developments. These techniques comprise confocal detection, multi-photon and pulsed excitation, laser scanning 3D imaging using piezo- and galvano-scanning units, stereological methods, image analysis as well as deconvolution algorithms, time-resolved imaging, Foerster resonance energy transfer (FRET) analysis, fluorescence recovery after photobleaching (FRAP), fluorescence loss in photobleaching (FLIP), single- and multi- channel fluorescence correlation spectroscopy (FCS), fluorescence lifetime correlation spectroscopy (FLCS), multi-focus fluorescence correlation spectroscopy and a variety of specialized single molecule fluorescence analysis methods.

Several of these techniques are either not yet fully developed or still are searching for applications in biological and medical sciences. Thus, one aim of this project is to further develop these techniques and to develop first relevant applications of these techniques. These issues are summarized in the aims V001, V002, and V004. They are actually assigned two work places located at the Academy of Sciences of the Czech Republic, the J. Heyrovsky Institute of Physical Chemistry (M. Hof) and the Institute of Physiology (L. Kubinova). The first partner is specialized on development and novel applications of advanced “single molecule” approaches like single- and multi-channel FCS, FLCS, and multi-focus FCS using pulsed excitation. Moreover, the J. Heyrovsky Institute of Physical Chemistry has outstanding expertise in the application of electrochemical methods for the understanding of the interaction of ions with model membranes (aim V003, V. Mareček). Thus, a further aim of this project is to combine the electrochemical expertise with the know-how in fluorescence, and to apply that combination on model systems and, finally, on living cells. The Institute of Physiology has long lasting experience in laser scanning 3D intensity imaging using confocal detection or 2-photon excitation and has significantly contributed to the development of stereological methods, image analysis as well as deconvolution algorithms. The individual activities related to those 4 work packages in 2007 were (for details please see “AKTIVITY USKUTEČNĚNÉ v roce 2007”):

- a) Determination of diffusion coefficients in biomembranes by z-scan FCS:
A comparison between one and two photon excitation
- b) Using FLCS as a unique and new tool for the simultaneous determination of diffusion coefficients in the two opposing leaflets of biomembranes
- c) FLCS as a new tool for the characterisation of protein-membrane interactions [1-4]
- d) FLCS as new tool for investigating the internal dynamics during DNA condensation [5-7]
- e) Testing of new condensing agents by FCS and FLCS [8]
- f) Characterisation of liposomes relevant to gene delivery [9-13]
- g) Development of methods for pre-processing of image data acquired by confocal and two-photon microscopy – stage 2
- h) Methods combining image analysis and stereological approach for evaluation of 3D microscopic image data – stage 2 [14-16]
- i) Analysis of data acquired by confocal and two-photon microscopy using different fluorescence microscopy techniques – stage 2
- j) Ion-pairing of phospholipids in the aqueous phase [17,18]
- k) Self-Organization of Water in Lithium/Nitrobenzene system [19]

It should be pointed out that, in most laboratories, expertise is restricted to some of these specialized techniques, and the full potential of advanced fluorescence microscopy in the investigated biological systems is only partially exploited. Moreover, biologists are often not aware of all possibilities in fluorescence microscopy and, thus, only in some exceptional cases cutting edge technologies are applied in answering questions in cell biology. This project aims to overcome these limitations by promoting the close collaboration of these two complementary fluorescence microscopy laboratories together practically covering the entire needed expertise in advanced microscopy with four teams formulating important questions in cell biology. These teams are headed by following scientists: P. Svoboda (Institute of Physiology), Z. Palkova (Faculty of Nature Sciences, Charles University), P. Hozak and J. Blahos (both Institute of Molecular Genetics, Academy of Sciences of the Czech Republic). All four teams are applying new strategies for controlled labeling of systems of interests with appropriate markers, and are performing fluorescence measurements on living cells in the laboratories equipped with those advanced fluorescence microscopy techniques. Moreover the know-how in cell handling was transferred to the laboratories headed by M. Hof and L.

Kubinova. These activities are directly connected to the aims V005 - V008 formulated in the original proposal. The individual activities aiming for the application of advanced fluorescence microscopy in biosciences in 2007 were (for details please see “AKTIVITY USKUTEČNĚNÉ v roce 2007”):

- a) Determination of detergent effect on steady-state anisotropy of DPH in plasma membrane preparation
- b) Functional activity of trimeric G proteins in preparations of detergent-resistant membrane domains (DRMs)
- c) Time-resolved FRET analysis of cell membrane proteins of interest
- d) The role of plasma membrane integrity in mechanism of hormone action and desensitization [20,21]
- e) Search for novel targets for proper positioning of small fluorophores within protein sequences and structures
- f) Investigate the intramolecular FRET between different fluorophores
- g) Monitoring nuclear translocation of NMI
- h) Dynamics and mechanism of nuclear import of NMI
- i) Monitoring mobility properties of NMI domains
- j) Identification of dynamics of PML interactions with nucleolus in senescent cells during recovery of rDNA synthesis inhibited by actinomycin D. [22]
- k) Preparation of the *Saccharomyces cerevisiae* strains containing Ato transporters fused with red fluorescent protein.
- l) FRET analysis of possible interaction of Ato-FP proteins
- m) Two-photon microscopy analysis of profile of Ato1p-GFP production in colonies of parental strain and in colonies of selected mutants
- n) Preparation of *S.cerevisiae* strains mutated in selected genes containing ATO1-GFP genomic fusions.
- o) Monitoring of production of cell wall protein Flo11p-GFP in structured colonies of Sigma1267 strain

Naturally, for the first 22 months of this 5 year project, there is still an imbalance regarding full papers coming from aims V001-V004 on one side (24) and from aims V005-V008 (3) on the other side. Activities connected to V001-V004 can be considered to a major extent to be ongoing projects of the individual laboratories located at the J.Heyrovsky Institute of Physical

Chemistry and the Institute of Physiology. On the other hand, the newly in the biological laboratories developed tools were to same extent not in publishable form without endangering the whole project, as the laborous finding of the appropriate mutants precedes their use, which we, in case of publication, fear might be used by our competitors. However, fluorescence experiments on those systems were performed and some of the results could already be included to full publications [20-22]. It can be expected that in 2008 the number of full publications in coming from those laboratories will further increase.

B. Other Activities in 2007

i) Students involved

PhD students: 7 (Hof) + 3 (Palkova) + 8 (Kubinova, Svoboda) + 3 (Blahos) + 9 (Hozák)

Mgr. or Ing. students: 3 (Hof) + 1 (Palkova) + 1 (Kubinova) + 2 (Hozák)

ii) Teaching Regular Courses at Universities

M. Hof: Fluorescence Spectroscopy in Biology and Medicine (Czech Technical University in Prague), Spectroscopy (University Olomouc)

Z Palková: Molecular Biology (“Molekulární biologie”, Faculty of Science, Charles University in Prague)

Cell Cycle and Signalling (“Buněčné cykly a signály”, Faculty of Science, Charles University in Prague)

A. Holoubek: Methods in molecular and cellular biology (“Seminář metod v molekulární a buněčné biologii”)

L. Kubínová: Quantitative Plant Anatomy (“Kvantitativní anatomie rostlin”, Faculty of Science, Charles University in Prague)

J. Blahoš: Courses of Pharmacology, 2nd medical School, Charles University, Prague

P. Hozák: The cell nucleus and regulation of gene expression („Buněčné jádro a regulace genové exprese“, Faculty of Science, Charles University in Prague)

P. Hozák and L. Kubínová: Microscopy image acquisition and processing (“Získání a zpracování mikroskopického obrazu”, PhD course, Faculty of Science, Charles University in Prague)

iii) Organized Conferences

P. Hozák (president), L. Kubínová (scientific secretary): 8th Multinational Congress on Microscopy, 17-21 June, Prague

iv) Invited Lectures at International Conferences, Seminars, Workshops, or Courses

M. Hof:

- „Principles and Applications of Fluorescence Lifetime Correlation Spectroscopy“
8th Multinational Congress on Microscopy, 17-21 June, Prague
- „Principles and Applications of Fluorescence Lifetime Correlation Spectroscopy“, Salzburg, ‘10th Conference on Methods and Applications of Fluorescence’, 11.9.2007 <http://www.maf-sip.com/maf10/conference-program-incl-lectures/>, 350 participants
- „Fluorescence Lifetime Correlation Spectroscopy“, at the Electrochemical Seminar Prague-Dresden, 15 -17 November 2007, Dresden, 30 participants

J. Humpolíčková:

- 13th International Workshop on Single Molecule Spectroscopy and, Berlin, 9/2007
- 5th Advanced Practical Course on Optical Spectroscopy in Biology , Juelich, 10/2007

L. Kubínová :

- XI School of Pure and Applied Biophysics “Advanced Optical Microscopy Methods in Biophysics”, Venice, Italy, January 29 – February 2, 2007

J. Blahos:

- Bordeaux PENS Training Center *European Synapse Summer School* September 2007

v) Obtained Academic Degrees

- Mgr.: R. Šachl, I. Ferčíková
- PhD’s: D. Durchánková, V. Rudajev, J. Stöhr
- Professorship: P. Hozák

vi) Web-side referring to this project

vii) Awards

- A. Bendu obtained two awards for his PhD:
 - a) “Cenu ministra školství, mládeže a tělovýchovy pro vynikající studenty a absolventy studia ve studijním programu”
 - b) “Česká hlava 2007 v kategorii Doctorandus”
- J. Humpolíčková obtained “Cenu Ucene společnosti v kategorii mlady vedecky pracovník do 35 let”
- Adam Miszta: First prize (500 Euro) on the European Biophysical Society best poster contest at the 2nd workshop on biophysics of membrane-active peptides, 1-4 April 2007 Lisbon, Portugal,

viii) Miscellaneous

P. Hozák serves as member of the executive committee of European Microscopy Society, and the president of the Czechoslovak Microscopy Society. There are several other scientific or pedagogic activities of the members of this project. However, it is certainly natural for active scientific groups that their members to present posters at international conferences, review research articles or proposals, organize regular seminars for students, invite international well recognized scientists for lectures or longer stays, or are invited by international well recognized work places for lectures or longer stays. Thus, we believe that it is not necessary to list those activities here in details.

C. Full publications acknowledging LC06063 (appeared, in press, or submitted in 2007)

- 1) Adam Miszta, Bas van Deursen, Roy Schoufs, Martin Hof, and Wim Th. Hermens "Absence of Ethanol-Induced Interdigitation in Supported Phospholipid Bilayers on Silica Surfaces" (2008) *Langmuir*, 24, 19-21. (IF=3.9)
- 2) Jan Sýkora, Karin Kaiser, Ingo Gregor, Wolfgang Bönigk, Günther Schmalzing, and Jörg Enderlein "Exploring Fluorescence Antibunching in Solution To Determine the Stoichiometry of Molecular Complexes" (2007) *Anal. Chem* 79, 4040-4049. (IF=5.6)

- 3) Adam Miszta, Radek Machan, Aleš Benda, Andre J Ouellette, Wim Th. Hermens, Martin Hof "Combination of ellipsometry, laser scanning microscopy and Z-scan fluorescence correlation spectroscopy elucidating interaction of cryptdin-4 with supported phospholipid bilayers" (2008) *Journal of Peptide Science*, in press. (IF=1.8)
- 4) Adam Miszta, Radek Macháň, Wim Th. Hermens, Martin Hof "Peptide-membrane interactions studied by ellipsometry, laser scanning and z-scan fluorescence correlation spectroscopy" kapitola knihy "Membrane-active peptides: methods and results on structure and function", in press.
- 5) Jana Humpolíčková, Aleš Benda, Jan Sýkora, Radek Macháň, Teresa Kral, Barbara Gasinska, Joerg Enderlein and Martin Hof "Equilibrium Dynamics of Spermine-induced Plasmid DNA Condensation Revealed by Fluorescence Lifetime Correlation Spectroscopy" (2007) *Biophys J*, 94(3), L17-9. (IF=4.8)
- 6) Jana Humpolíčková, Aleš Benda, Martin Hof "The compaction mechanism of an intermediate-sized DNA molecule elucidated by fluorescence lifetime correlation spectroscopy" *Chemické listy*, submitted. (IF=0.4)
- 7) Jana Humpolíčková, Miroslav Štěpánek, Teresa Kral, Aleš Benda, Karel Procházka, Martin Hof "On Mechanism of Intermediate-sized Circular DNA Compaction Mediated by Spermine: Contribution of Fluorescence Lifetime Correlation Spectroscopy" *Journal of Fluorescence*, in press. (IF=2.6)
- 8) Noppadon Adjimatera, Aleš Benda, Ian S. Blagbrough, Marek Langner, Martin Hof, Teresa Kral "Fluorescence Correlation Spectroscopic Studies of a Single Lipopolyamine-DNA Nanoparticle" (2008) kapitola z knihy "Fluorescence of Supermolecules, Polymers, and Nanosystems", Springer Ser Fluoresc, 4, 381-413.
- 9) Agnieszka Olżyńska, Piotr Jurkiewicz, Martin Hof "Properties of Mixed Cationic Membranes studied by Fluorescence Solvent Relaxation" *Journal of Fluorescence*, in press. (IF=2.6)
- 10) Rieber K, Sýkora J, Olżyńska A, Jelinek R, Cevc G, Hof M "The use of solvent relaxation technique to investigate headgroup hydration and protein binding of simple and mixed phosphatidylcholine/surfactant bilayer membranes" (2007) *Biochim. Biophys. Acta*, 1768, 1050-1058. (IF=3.6)
- 11) A. Olżyńska, A. Zan, P. Jurkiewicz, J. Sýkora, G. Gröbner, M. Langner, M. Hof "Molecular interpretation of fluorescence solvent relaxation of Patman and 2H NMR experiments in phosphatidylcholine bilayers" (2007) *Chem. Phys. Lipids*, 147, 69-77. (IF=2.4)

- 12) Sýkora Jan, Slavíček Petr, Jungwirth Pavel, Barucha Justyna, Hof Martin "Time-Dependent Stokes Shifts of Fluorescent Dyes in the Hydrophobic Backbone Region of a Phospholipid Bilayer: Combination of Fluorescence Spectroscopy and Ab Initio Calculations" (2007) *Journal of Physical Chemistry B*, 111, 5869-5877. (IF=4.1)
- 13) Blanco-Rodríguez Ana Maria, Ronayne Kate L., Záliš Stanislav, Sýkora Jan, Hof Martin, Vlček Antonín Jr. "Solvation-Driven Excited-State Dynamics of [Re(4-Et-pyridine)(CO)₃(2,2'-bipyridine)]⁺ in Imidazolium Ionic Liquids. A Time-Resolved Infrared and Phosphorescence Study" *Journal of Physical Chemistry A*, in press. (IF=3.0)
- 14) Albrechtová, J., Janáček, J., Lhotáková, Z., Radochová, R., Kubínová, L.: Novel efficient methods for measuring mesophyll anatomical characteristics from fresh thick sections using stereology and confocal microscopy: application on acid rain-treated Norway spruce needles. *Journal of Experimental Botany* 58 (6): 1451-1461, 2007 (IF=3,630).
- 15) Čebašek, V., Kubínová, L., Janáček, J., Ribarič, S., Eržen, I.: Adaptation of muscle fibre types and capillary network to acute denervation and shortlasting reinnervation. *Cell and Tissue Research* 330 (2): 279-289, 2007 (IF=2,580).
- 16) Kubínová, L., Kutík, J.: Surface density and volume density measurements of chloroplast thylakoids in maize (*Zea mays* L.) under chilling conditions. *Photosynthetica* 45(4): 481-488, 2007. (IF=0,782)
- 17) Hana Jänchenová, Alexandr Lhotský, Karel Štulík, Vladimír Mareček Adsorption and Ion-pairing Interactions of Phospholipids in the System of Two Immiscible Electrolyte Solutions. Part I. The Behaviour of Lecithin at the Water/1,2-Dichloroethane Interface, Compared with that of Trimethyloctadecylammonium Cation. *J. Electroanal. Chem.*, 601 101 (2007). (IF=2,3).
- 18) H. Jänchenová, K. Štulík, V. Mareček, Adsorption and Ion-pairing Interactions of Phospholipids in the System of Two Immiscible Electrolyte Solutions. Part II. Formation and Behaviour of a Lecithin Layer in the Presence of Multivalent Anions in the Aqueous Phase, *J. Electroanal. Chem.*, 604 109 (2007). (IF=2,3).
- 19) Greg Moakes, Leslie T. Gelbaum Johannes Leisen, Jiri Janata, Vladimír Mareček, Luc L. Daemon, Self-organization of water in lithium/nitrobenzene system, *J. Phys. Chem. B*, 111 7312 (2007). (IF=4.1)
- 20) Ostašov, P., Bourova, L., Hejnova, L., Novotny, J. and Petr Svoboda (2007), Disruption of the plasma membrane integrity by cholesterol depletion impairs

effectiveness of TRH receptor-mediated signal transduction via Gq α /G11 α proteins. J Recept Signal Transduct Res. 27, 335-352 (IF=2.0)

- 21) D. Durchánková and P. Svoboda (2007). Time-course of agonist-induced solubilization of trimeric Gq α /G11 α proteins resolved by two-dimensional electrophoresis. Physiol Res. 2007 [Epub ahead of print], in press (IF=2,1)..
- 22) Janderová-Rossmeislová L, Nováková Z, Vlasáková J, Philimonenko V, Hozák P, Hodný Z.: PML protein association with specific nucleolar structures differs in normal, tumor and senescent human cells. J Struct Biol., 2007, 159, 56-70. (IF=3.5).

D. Full publications acknowledging LC06063 appeared in 2006

- 23) Magdalena Przybylo, Jan Sýkora, Jana Humpolíčková, Aleš Benda, Anna Zan, Martin Hof „The lipid diffusion in giant unilamellar vesicles is more than two times faster than in supported phospholipid bilayers under identical conditions“ (2006) Langmuir, 22, 9096-9099. (IF=3.9)
- 24) Humpolickova J, Gielen E, Benda A, Fagulova V, Vercammen J, Vandeven M, Hof M, Ameloot M, Engelborghs Y, „Probing diffusion laws within cellular membranes by Z-scan fluorescence correlation spectroscopy“ (2006) Biophysical Journal, 91(3), L23-25. (IF=4.8)
- 25) Aleš Benda, Veronika Fagul'ová, Alexander Deyneka, Joerg Enderlein and Martin Hof „Fluorescence Lifetime Correlation Spectroscopy Combined with Lifetime Tuning: New Perspectives in Supported Phospholipid Bilayer Research“ (2006) Langmuir, 22, 9580-9585 (IF=3.9)
- 26) P. Jurkiewicz, A. Olżyńska, M. Langner, M. Hof „Headgroup Hydration and Mobility of DOTAP/DOPC Bilayers: A Fluorescence Solvent Relaxation Study“ (2006) Langmuir, 22, 8741-8749 (IF=3.9)
- 27) ČAPEK, M.; JANÁČEK, J.; KUBÍNOVÁ, L. Methods for compensation of the light attenuation with depth of images captured by a confocal microscope. Microscopy Research and Technique, August 2006, vol. 69, no.8, s. 624-635. (IF=1.7)