

Progress Report for 2009

(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 Prof. Dr. rer.nat., DSc.
PARTICIPANTS	Kubínová Lucie RNDr. CSc. Hozák Pavel Prof. RNDr. DrSc. Palková Zdena Prof.RNDr. CSc.
ASSOCIATED PARTICIPANTS	Mareček Vladimír Prof. Ing. DrSc. Svoboda Petr Doc. RNDr. DrSc. Blahoš Jaroslav MUDr. Ph. Doc.
NUMBER OF APPEARED AND ACCEPTED FULL PUBLICATIONS WITHIN FIRST 46 MONTHS	62 (average impact factor 3.3)
OBTAINED ACADEMIC DEGREES WITHIN FIRST 46 MONTHS	27 (2 BC, 7 MGR, 12 PHD, 1 DOC, 2 DSC, 3 PROF.)

A. Overview on Scientific Activities in 2009

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 improvements. 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. Methods for enhancement of resolution in microscopy above the diffraction limit have been developed recently and one of the methods (DSOM) has been implemented in the laboratory of prof. Hof [2].

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 their first relevant applications. These issues are summarized in the aims V001, V002, and V004 in the original grant proposal (see contract pages 8-18). They are actually assigned to two work places located at the Academy of Sciences of the Czech Republic, the J.Heyrovský 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.Heyrovský 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 2009 were (for details please see “AKTIVITY USKUTEČNĚNÉ v roce 2009” in the report:

- a) Using FLCS as an unique and new tool for the simultaneous determination of diffusion coefficients in the two opposing leaflets of biomembranes addressing lipid asymmetry- stage 3 [13]
- b) Characterisation of protein (peptide)-membrane interactions-stage 3: Combination of Ellipsometry, laser scanning microscopy, Z-scan fluorescence correlation spectroscopy, FLCS, and fluorescence anti-bunching experiments [5,10,12]
- c) Testing of new condensing agents and DNA carriers by FCS, FLCS and solvent relaxation technique- stage 3 [1,9,14]
- d) Establishing Energy transfer as a tool for determination of co-localisation in membranes of living cells and model membranes-stage 2 [6,8,11]
- e) Application of advanced fluorescence techniques in the elucidation of the structure function relationship of dehalogenase proteins [3,7]
- f) Development and application of spectrally resolved fluorescence confocal microscope [2] (all Hof)
- g) Proton transfer across a liquid|liquid interface facilitated by phospholipid interfacial films. [15] (Mareček)
- h) Development of methods for pre-processing of image data acquired by confocal and two-photon microscopy - stage 4
- i) Methods combining image analysis and stereological approach for evaluation of 3D microscopic image data – stage 4 [20,21,24-27]
- j) Analysis of data acquired by confocal and two-photon microscopy using different fluorescence microscopy techniques – stage 4 [16,22,23,26] (all Kubinova)

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 investigation of 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 the current 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 (see contract pages 8-18). The individual activities aiming for the application of advanced fluorescence microscopy in biosciences in 2009 were (for details please see “AKTIVITY USKUTEČNĚNÉ v roce 2009” in the report:

- a) The influence of cholesterol depletion on TRH-receptor mobility in plasma (cell) membrane of living cells
- b) The effect of cholesterol depletion on functional parameters of DOR-Gi coupling. Correlation with structural and dynamic characteristics of hydrophobic membrane interior.
- c) Agonist-stimulated G protein activity in plasma membrane compartments isolated from rat brain cortex [18]
- d) Receptor-G-protein coupling efficiency in detergent-resistant membrane domains [3] (all Svoboda)

- e) Comparison of YFP-CFP and CFP-FlAsH fluorophor pairs in detection of receptor activation
- f) Intestinal cell-specific vitamin D receptor (VDR)-mediated transcriptional regulation of CYP3A4 gene [19]
- g) CFP based evaluation of metabotropic glutamate receptor-G protein relations using CFP (Blahoš)

- h) FRET analysis of interaction of Ato-FP proteins - stage 3
- i) Development of codon-optimized pH- and redox-sensitive GFP probes for in vivo organellar measurements. Stage 2
- j) Confocal microscopy with GFP fusions and fluorescent potentiometric probes.

- k) Architecture of developing multicellular yeast colony: spatio-temporal expression of Ato1p ammonium exporter. [17]
- l) Two-photon microscopy analysis of profile of Ato1p-GFP production in colonies of parental strain and in colonies of selected mutants - stage 3 [16] (all Palkova)
- m) Determining the way myosins travel to the nucleus
- n) Describing dynamic properties of two nuclear isoforms of the myosin 1C.
- o) Evaluation of dynamic properties of nuclear myosin connected with actin binding. (all Hozak)

After 46 month of solving the project, there still exists an imbalance between the numbers of publications addressing aims V001-V004 on one side and aims V005-V008 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, while activities connected with aims V005-V008 result from the collaborations established within the centre and to a large extent make use of the results of aims V001-V004. That is the main reason why the advance in activities connected with aims V005-V008 seems slower. Nevertheless there have already appeared publications resulting from collaborations established within the center, namely between the laboratories of prof. Hof and doc. Svoboda and between the laboratories of prof. Palková and Dr. Kubínová. There also exist an amount of results of activities connected with aims V005-V008 resulting from those collaborations, which has not been published yet, but represents a promise of further publications in the next few years.

B. Other Activities

i) Students involved in 2009

PhD students: 7 (Hof) + 3 (Palkova) + 2 (Kubinova) + 7 (Svoboda) + 3 (Blahoš) + 4 (Hozák)

Mgr. or Ing. students: 2 (Hof) + 2 (Palkova) + 2 (Svoboda) + 1 (Blahoš) + 2 (Hozák)

ii) Teaching Regular Courses at Universities in 2009

M. Hof: Fluorescence Spectroscopy in Biology and Medicine (half of the course at Czech Technical University in Prague and South-Bohemian University České Budějovice), Spectroscopy (University Olomouc) and Fluorescence Spectroscopy (Charles University in Prague, Faculty of Science)

J. Sýkora: Fluorescence Spectroscopy in Biology and Medicine (half of the course at South-Bohemian University České Budějovice)

R. Machan: Fluorescence Spectroscopy in Biology and Medicine (half of the course at Czech Technical University in Prague)

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 and J. Janáček: Image analysis and stereological methods for biologists („Metody analýzy obrazu a stereologie pro biology“, Faculty of Science, Charles University in Prague)

J. Blahoš: Medical pharmacology, 2nd Medical School Charles University in Prague and Medical Faculty Charles University in Hradec Kralove

P. Hozák: Microscopy methods in three PhD courses (Faculty of Science, Charles University in Prague)

P. Svoboda: Molecular Pharmacology (“Přednášky a praktická cvičení: vazebné studie s radioligandy, podbuněčná frakcinace tkání a buněčných liní, základy práce s isotopy, demonstrace s využitím fluorescenčních technik).

iii) Organized Conferences in 2009

iv) Invited Lectures at International Conferences, Seminars, Workshops, or Courses in 2008

M. Hof:

- Invited lecture „Hydration and mobility in membranes and enzymes by the solvent relaxation technique“ at the international conference „Regional Biophysics Conference 2009 Linz “ (Linz, 11.-13.2. 2009). (M.Hof)

- Invited lecture „Hydration and mobility in membranes and enzymes by the solvent relaxation technique“ at the international conference „FluoFest 2009 Prague “ (Prague, 3.-5.6. 2009). (M.Hof)
- Invited lecture „ Fluorescence Lifetime Correlation Spectroscopy.“ at the international conference „ 15th Anniversary International Workshop on Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences Berlin, September 15-18, 2009. (M. Hof)
- Invited lecture „Hydration and mobility close to the active site of enzymes determined by the solvent relaxation technique: Elucidating the dynamics - function relationship.“ at the conference „Mini-symposium on computation of interactions in biological systems “ (Nové Hradky, 10.-13.12. 2009). (J. Sykora)
- Invited lecture „Hydration and mobility close to the active site of enzymes determined by the solvent relaxation technique: Elucidating the dynamics - function relationship.“ at the international conference „11th International Conference on Methods and Applications of Fluorescence: Spectroscopy, Imaging and Probes“ (Budapest, 6.-9.9. 2009). (J. Sykora)
- Invited lecture „Spectral Fluorescence Correlation Spectroscopy.“ at the international conference „11th International Conference on Methods and Applications of Fluorescence: Spectroscopy, Imaging and Probes“ (Budapest, 6.-9.9. 2009). (A. Benda)
- Invited lecture „Spectral Fluorescence Correlation Spectroscopy.“ at the international conference „ 15th Anniversary International Workshop on Single Molecule Spectroscopy and Ultra Sensitive Analysis in the Life Sciences Berlin, September 15-18, 2009. (A. Benda)

L.Kubínová:

- Invited lecture: “Systematic sampling in 3D using virtual test probes and its application to surface area and length measurement”. 10th European Congress of ISS on Stereology and Image Analysis, Milan, June 22-26, 2009.
- Invited lecture: “Stereological methods for estimating geometrical characteristics of biological structures using virtual test probes applied to 3D images”. Interdisciplinary Symposium on 3D microscopy 2009 in Interlaken, July 12-16, 2009.
- Invited lecture: “Current 3D microscopy and deep tissue imaging”. Výjezdní zasedání doktorského grantu 204/09/H084, Průhonice, 5.11. 2009.

J. Janáček:

- Invited lecture: “Variances of volume, surface area and length estimates by 3D virtual grids”. 10th European Congress of ISS on Stereology and Image Analysis, Milan, June 22-26, 2009.

O. Chernyavskiy:

- Invited lecture: “Practical notes on fluorescence lifetime imaging”. 5. Metodické dny, hotel Petr Bezruč, Beskydy, 11.-14. října 2009.

P. Hozák:

- Invited lecture: “Nuclear myosin 1 – the muscles for DNA transcription?”, National Institute of Genetics, Mishima, Japan

J. Blahoš:

- Invited lecture: Jaroslav Blahoš“ Assymetric signalling of dimeric Class C GPCRsESF exploratory workshop on “GPCR Signalling Systems: A New Avenue For Drug Discovery?” 24-25/11/2009

Z. Palková:

- Invited lecture: (2009) Life within a multicellular community: Yeast colony architecture and physiology. John Hopkins University School of Medicine, Baltimore, MD, USA

v) **Obtained Academic Degrees**

- Bc.: M. Kolářová, A. Vojtíšková
- Mgr./Ing.: T. Steinberger, O. Hruboš, J. Brejchová
- PhD: A. Miszta,
- Professorship: M. Hof

vi) **Web-site referring to this project**

<http://www.jh-inst.cas.cz/~fluorescence/NRC.htm>

vii) **Awards**

- Dr. J. Sýkora obtained “Cenu Josefa Hlávky v kategorii mladý vědecký pracovník do 33 let”
- Karel Preis Prize 2009 for authors: L.Beranová, J.Humpolíčková and M. Hof for "Fluorescence Corelation Spectroscopy", the best communication published in 2009 in *Chemické Listy (Chem. listy, 103, 125-129, 2009)*

viii) **Miscellaneous**

P. Hozák serves as member of the executive committee of European Microscopy Society, and the president of the European “Society for Histochemistry,” and Czechoslovak Microscopy Society. Martin Hof was appointed as main editor for Europe of Journal of Fluorescence. J. Blahoš is in committee of Sigma-Aldrich Conference for Young Chemists and Biochemists. P. Svoboda is head of Laboratory of Neurobiology, Department of Physiology, Faculty of Science, Charles Univeristy.

Moreover, 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 (20), in press (6), or submitted (1) in 2009)

- 1) Humpolickova J, Benda A, Beranova L, Hof M. Compaction mechanism of intermediate-sized dna elucidated by fluorescence lifetime correlation spectroscopy. *Chemicke Listy* 2009; 103(11):911-914. (IF=0.6)
- 2) Humpolickova J, Benda A, Enderlein J. Optical Saturation as a Versatile Tool to Enhance Resolution in Confocal Microscopy. *Biophysical Journal* 2009; 97(9):2623-2629. (IF=4.7)
- 3) Sykora J, Bourova L, Hof M, Svoboda P. The effect of detergents on trimeric G-protein activity in isolated plasma membranes from rat brain cortex: Correlation with studies of DPH and Laurdan fluorescence. *Biochimica et Biophysica Acta-Biomembranes* 2009; 1788(2):324-332. (IF=4.2)
- 4) Jesenska A, Sykora J, Olzynska A, Brezovsky J, Zdrahal Z, Damborsky J, et al. Nanosecond Time-Dependent Stokes Shift at the Tunnel Mouth of Haloalkane Dehalogenases. *Journal of the American Chemical Society* 2009; 131(2):494-501. (IF=8.1)
- 5) Stefl M, Kulakowska A, Hof M. Simultaneous Characterization of Lateral Lipid and Prothrombin Diffusion Coefficients by Z-Scan Fluorescence Correlation Spectroscopy. *Biophysical Journal* 2009; 97(3):LO1-LO3. (IF=4.7)
- 6) Sachl R, Mikhalyov I, Hof M, Johansson LBA. A comparative study on ganglioside micelles using electronic energy transfer, fluorescence correlation spectroscopy and light scattering techniques. *Physical Chemistry Chemical Physics* 2009; 11(21):4335-4343. (IF=4.0)
- 7) Blanco-Rodriguez AM, Busby M, Ronayne K, Towrie M, Gradinaru C, Sudhamsu J, et al. Relaxation Dynamics of Pseudomonas aeruginosa Re-I(CO)(3)(alpha-diimine)(HisX)(+) (X=83, 107, 109, 124, 126)Cu-II Azurins. *Journal of the American Chemical Society* 2009; 131(33):11788-11800. (IF=8.1)

- 8) Huranova M, Jablonski JA, Benda A, Hof M, Stanek D, Caputi M. In vivo detection of RNA-binding protein interactions with cognate RNA sequences by fluorescence resonance energy transfer. *RNA-A Publication of the RNA Society* 2009; 15(11):2063-2071. (IF=5.0)
- 9) Olzynska A, Jurkiewicz P, Hof M. Fluorescence solvent relaxation in cationic membranes. In: Geddes CD, editor. *Reviews in Fluorescence* 2007. New York: Springer; 2009. p. 119-138.
- 10) Miszta A, Machan R, Hermens WT, Hof M. Peptide-membrane interactions studied by ellipsometry, laser scanning microscopy and z-scan fluorescence correlation spectroscopy. In: Castanho MARB, editor. *Membrane Active Peptides: Methods and Results on Structure and Function* La Jolla: International University Line; 2009. p. 227-256.
- 11) Machan R, Hof M. Fluorescence Spectroscopy. In: Vo-Dinh T, Gauglitz G, editors. *Handbook of Spectroscopy*. 2nd ed. Weinheim: Wiley InterScience; 2009. in press.
- 12) Macháň R, Miszta A, Hermens W, Hof M. Real-time monitoring of melittin induced pore and tubule formation from supported lipid bilayers and its physiological relevance. *Chemistry and Physics of Lipids* 2009, doi: 10.1016/j.chemphyslip.2009.11.005. (IF=2.6)
- 13) Kulakowska A, Jurkiewicz P, Sykora J, Benda A, Mely Y, Hof M. Fluorescence Lifetime Tuning—A Novel Approach to Study Flip-Flop Kinetics in Supported Phospholipid Bilayers. *Journal of Fluorescence* 2009, doi: 10.1007/s10895-009-0581-9. (IF=1.9)
- 14) Barucha-Kraszewska J, Kraszewski S, Jurkiewicz P, Ramseyer C, Hof M. Numerical studies of the membrane fluorescent dyes dynamics in ground and excited states. *Biochimica et Biophysica Acta-Biomembranes* 2009; submitted. (IF=4.2)
- 15) Holub K, Janchenova H, Stulik K, Marecek V. Proton transfer across a liquid/liquid interface facilitated by phospholipid interfacial films. *Journal of Electroanalytical Chemistry* 2009; 632(1-2):8-13. (IF=2.5)
- 16) Vachova L, Chernyavskiy O, Strachotová D, Bianchini P, Burdiková Z, Ferciková I, Kubinova L, Palkova Z (2009) Architecture of developing multicellular yeast colony: spatio-temporal expression of Ato1p ammonium exporter. *Environ Microbiol*. 11: 1866-1877. (IF = 4.7)
- 17) Vopalenska I, Stovicek V, Janderova B, Vachova L, Palkova Z (2010), Role of distinct dimorphic transitions in territory colonizing and formation of yeast colony architecture. *Environ Microbiol*,12, 264–277. (IF = 4.7)
- 18) Bourova, L., Stöhr, J., Lisy, V., Rudajev, V., Novotny, J. and Svoboda, P. (2009) G-protein activity in percoll-purified plasma membranes, bulk plasma membranes and low-

- density plasma membranes isolated from rat cerebral cortex. *Medical Science Monitor* 15(4), BR111-122 (IF=1.607)
- 19) Pavěk P, Pospechová K, Svecová L, Syrová Z, Stejskalová L, Blázková J, Dvůrak Z, Blahos J. Intestinal cell-specific vitamin D receptor (VDR)-mediated transcriptional regulation of CYP3A4 gene *Biochem Pharmacol.* 2010 Jan 1579(2):277-87. Epub 2009 Aug 25. IF=4,838
- 20) Karen, P., Števanec, M., Smerdu, V., Cvetko, E., Kubínová, L., Eržen, I.: Software for muscle type classification and analysis. *European Journal of Histochemistry* 53(2): 87-95, 2009. (IF = 1.6)
- 21) Čebašek, V., Eržen, I., Vyhnal, A., Janáček, J., Ribarič, S., Kubínová, L.: The estimation error of skeletal muscle capillary supply is significantly reduced by 3D method. *Microvascular Research* 79(1): 40-46, 2010. (IF = 3.0)
- 22) Griffiths, P.J., Isackson, H., Pelc, R., Redwood, C.S., Funari, S.S., Watkins, H., Ashley C.C.: Synchronous In Situ ATPase Activity, Mechanics, and Ca²⁺ Sensitivity of Human and Porcine Myocardium. *Biophysical Journal* 97(9): 2503-2512, 2009. (IF = 4.683)
- 23) Pelc, R., Ishii, N., Ashley, C. C.: Laser flash photolysis of diazo-2, a caged calcium chelator: The relationship between the extent and rate of smooth muscle relaxation. *Journal of Laser Applications* 21(1): 32-38, 2009. (IF = 0.549)
- 24) Vohník, M., Burdík, Z., Albrechtová, J., Vosátka, M.: Testate amoebae (Arcellinida and Euglyphida) vs. ericoid mycorrhizal and DSE fungi: A possible novel interaction in the mycorrhizosphere of ericaceous plants? - *Microbial Ecology* 57: 203-214, 2009. (IF=2.9)
- 25) Čápek, M., Brůža, P., Janáček, J., Karen, P., Kubínová, L., Vágnerová, R.: Volume reconstruction of large tissue specimens from serial physical sections using confocal microscopy and correction of cutting deformations by elastic registration. *Microscopy Research and Technique*.72: 110-119, 2009. (IF=1.8)
- 26) Chernyavskiy, O., Vannucci, L., Bianchini, P., Difato, F., Saieh, M., Kubínová, L.: Imaging of mouse experimental melanoma in vivo and ex vivo by combination of confocal and nonlinear microscopy. *Microscopy Research and Technique* 72: 411-423, 2009. (IF=1.8)
- 27) Janáček, J., Čebašek, V., Kubínová, L., Ribarič, S., Eržen, I.: 3D visualization and measurement of capillaries supplying metabolically different fiber types in the rat extensor digitorum longus muscle during denervation and reinnervation. *Journal of Histochemistry & Cytochemistry* 57(5): 437-447, 2009. (IF=2.8)

D. Full publications acknowledging LC06063 appeared in 2008

- 28) 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)
- 29) 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*, 14 (4): 503-509.(IF=1.8)
- 30) 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" (2008) *Biophys J*, 94(3), L17-9. (IF=4.8)
- 31) 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*, 18(3-4, 679-684 (IF=2.6)
- 32) Humpolickova, J., L. Beranova, M. Stepanek, A. Benda, K. Prochazka, and M. Hof, Fluorescence Lifetime Correlation Spectroscopy Reveals Compaction Mechanism of 10 kbp and 49 kbp DNA and Differences between Polycation and Cationic Surfactant. *Journal of Physical Chemistry B*, 2008, 112, 16823-16829. (IF=4.1)
- 33) 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.
- 34) Piotr Jurkiewicz, Čestmír Koňák, Vladimír Šubr, Martin Hof, Petr Štěpánek, Karel Ulbrich. Investigation of nanoparticle coating by fluorescence correlation spectroscopy. *Macromolecular Chemistry and Physics*, 2008, Roč. 209, č. 14, s. 1447-1453. (IF=2.0)
- 35) Agnieszka Olżyńska, Piotr Jurkiewicz, Martin Hof "Properties of Mixed Cationic Membranes studied by Fluorescence Solvent Relaxation" *Journal of Fluorescence*, 18(5), 925-928 (IF=2.6)

- 36) 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*, *Journal of Physical Chemistry A*, 2008, 112(16), 3506-3514 (IF=3.0)
- 37) Valenta, J., Fucikova, A., Vacha, F., Adamec, F., Humpolickova, J., Hof, M., Pelant, I., Kusova, K., Dohnalova, K., Linnros, J. *Advanced Functional Materials*, 2008, 18(18): 2666-2672 (IF=7.5)
- 38) Kratochvílová, Irena; Nešpůrek, Stanislav; Šebera, Jakub; Záliš, Stanislav; Pavelka, Matěj; Wang, G.; Sworakowski, J. New organic FET-like photoactive device, experiments and DFT modeling. *European Physical Journal E*, 2008, 25, 299-307.(IF=2.0)
- 39) Kubát, Pavel; Lang, Kamil; Lhoták, P.; Janda, Pavel; Sýkora, Jan; Matějčíček, P.; Hof, Martin; Procházka, K.; Zelinger, Zdeněk. Porphyrin/calixarene self-assemblies in aqueous solution. *Journal of Photochemistry and Photobiology. A - Chemistry Section*, 2008, 198, 18-25.(IF=1.9)
- 40) Durchánková, D., Novotny, J. and Svoboda, P. (2008) Time-course of agonist-induced solubilization of trimeric Gq/G11 alpha proteins resolved by two-dimensional electrophoresis. *Phys. Res.* 57, 195-203 (IF=1.5)
- 41) H. Jänchenová, K. Štulík, V. Mareček, Adsorption and ion-pairing interactions of phospholipids in the system of two immiscible electrolyte solutions - Part III: The behaviour of a lecithin layer at the water/1,2-dichloroethane interface at interfacial potential differences lower than the zero-charge potential difference, *J. Electroanal. Chem.* 612 186 (2008).(IF=2.6)
- 42) K. Maeda, T. Maekawa, Y. Yoshida, T. Okugaki, S. Kihara, V. Mareček, Synergistic barrier effect of phosphatidylcholine and phosphatidic acid on the ion transfer across a polarized liquid-liquid interface and its electrochemical stability, *J. Electroanal. Chem.* 619-620 53 (2008).(IF=2.6)
- 43) Pelc, R., Hostounský, Z., Otaki, T.: Correlation between off-axis illumination and apodized phase-contrast: two complementary microscopic phase-imaging modes. *Journal of Biomedical Optics* 13 (5): 054067, 2008. ISSN 1083-3668.(IF=3.0)
- 44) Jirkovská, M., Janáček, J., Kaláb, J., Kubínová, L.: Three-dimensional analysis of capillary bed and its angiogenic activity in terminal villi of normal term placenta. *Placenta* 29(10): 892-897, 2008. ISSN 0143-400. (IF=3.2)

- 45) Kumpost J, Syrova Z, Kulihova L, Frankova D, Bologna JC, Hlavackova V, Prezeau L, Kralikova M, Hruskova B, Pin JP, Blahos J. Surface expression of metabotropic glutamate receptor variants mGluR1a and mGluR1b in transfected HEK293 cells. *Neuropharmacology*, **55**:409-418, 2008 (IF=3.2)

E. Full publications acknowledging LC06063 appeared in 2007

- 46) 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)
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