2.2.1. AKTIVITY USKUTEČNĚNÉ v roce 2008

Číslo aktivity
LK01-8
Ke kterému dílčímu cíli se aktivita vztahuje
V004 - Vývoj nových metod pro předzpracování a analýzu obrazových dat získaných konfoká...
Název (cíl)aktivity
Development of methods for pre-processing of image data acquired by confocal and two-photon microscopy – stage 3
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
We have evaluated performance of several confocal microscopes using special test specimens, especially uniform thin fluorescent layers and fluorescent microspheres or ordinary coverslips (Chernyavskiy and Kubínová, 2008). For improving the possibilities of image acquisition of large specimens by a confocal microscope, we have developed a new method for automatic merging of overlapping fields of view using a motorized microscopic stage and an improved algorithm for compensation of the light attenuation with depth of images captured by a confocal microscope using MRF deformation model and graph cuts (Čapek et al., 2008). Further, we have optimized the newly installed Huygens software for deconvolution of images acquired by our confocal microscopy systems and improved our software for processing confocal image data, namely Link-MRC, Glue-MRC, and also image format conversion programme ConvMRC, as well as a software interface for loading 2D and 3D data acquired by various confocal microscopes into the Ellipse SW environment which we are using for many of our software applications. Persons involved: Chernyavskiy, Čapek, Karen, Janáček, Spudilová, Kubínová
Skutečné Indikátory dosažení - výsledky aktivity
The tests for calibration of our confocal microscope systems are prepared and we are using them on a regular basis for optimizing performance of our microscopes. The developed image processing methods and their software implementations are available for optimized image acquisition and processing.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Presentation of achieved results at international congresses and their publication. 4x authorized software (already included into RIV database).
1.Chernyavskiy, O., Kubínová, L.: Testing calibration standards for confocal and two-photon microscopy. Proceedings of 14th European Microscopy Congress, Aachen, September 1-5, 2008, Vol. 3, Heidelberg: Springer, pp.201-202, 2008.ISBN 978-3-540-85154-7
2.Čapek, M., Michálek, J., Janáček, J., Kubínová, L., Hána, K., Smrčka, P.: Compensation of the light attenuation with depth of images captured by a confocal microscope using a MRF deformation model and graph cuts. Eurographics "08, Heraklion, 14-18 April,2008, The Eurographics Association, pp. 307-310, 2008.ISSN 1017-4656
Authorized software:
1.Karen, P.: LinkMRC – user software for optimalized composition of consecutive stacks of images from confocal microscope (2008). RIV/67985823/08:00308637
2.Karen, P.: GlueMRC - user software for optimalized merging of mosaic-layout stacks of

images from confocal microscope (2008). RIV/67985823/08:00308640
3.Karen, P.: ConvMRC - user software for mutual conversion between stacks of images from confocal microscope and common graphic file formats (2008). RIV/67985823/08:00308642
4.Karen, P.: User software interface for loading 2D/3D data captured by various confocal microscopes into laboratory imaging system Ellipse (2008). RIV/67985823/08:00308644

Číslo aktivity
LK02-8
Ke kterému dílčímu cíli se aktivita vztahuje
V004 - Vývoj nových metod pro předzpracování a analýzu obrazových dat získaných konfoká...
Název (cíl)aktivity
Methods combining image analysis and stereological approach for evaluation of 3D microscopic image data – stage 3
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
We have developed new stereological and image analysis techniques for evaluation of 3D image data from different biological specimens with the aim to obtain biologically important geometrical parameters, namely in the studies of 3D arrangement of capillaries in terminal villi of human placenta (Jirkovská et al., 2008), capillaries supplying metabolically different fiber types in the rat extensor digitorum longus muscle (Janáček et al., accepted to Journal of Histochemistry & Cytochemistry), and microscopic structure of testate amoebae (Vohník et al., 2009, Burdíková 2008a,b,c). Spatial statistics methods for evaluation of mutual relationships of different tissue, cell and nuclear compartments based on 3D image data were developed and implemented in Mathematica SW (Vyhnal, 2008). New methods for volume reconstruction of large biological specimens, captured by confocal microscopy were developed, including a novel approach enabling to keep true object morphology using a priori information about the shape and size of the specimen, available from images of the cutting planes captured by a USB light microscope immediately before cutting the specimen by a microtome (Čapek et al., accepted to Microsc. Res. Techn., Čapek et al., 2008b,e). The errors of thus obtained 3D reconstructions from confocal microscopic images were evaluated using stereological measurements and compensated (Čapek et al., 2008a,c,d). The methods for surface area measurement based on 3D microscopic images were tested on geometrical models and different types of biological surfaces, namely tobacco cell walls, internal surface of conifer needles and walls of rat skeletal muscle fibres (Kubínová and Janáček, 2008, Kubínová, 2008). Persons involved: Čapek, Kubínová, Burdíková, Janáček, Vyhnal, Spudilová, Karen.
Skutečné Indikátory dosažení - výsledky aktivity
New relevant software implementations of the developed methods are available. Stereological and image analysis measurements were made, and images with relevant 3D reconstructions were prepared.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Achieved results were presented at international congresses, in invited lectures by Dr. Kubínová at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden and at the

Department of Mathematics, University of Milano, and published (or accepted for publication) in four peer-reviewed journals, i.e. Placenta (IF=3.238), Microscopy Research and Technique (IF=1.644), Microbial Ecology (IF=2.558), and Journal of Histochemistry & Cytochemistry (IF=2.335).

Papers in peer-reviewed journals:

1. 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=3.238
2. Vohník, M., Burdíková, 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.ISSN: 0095-3628.IF=2.558
3. Čapek, 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. - accepted for publication in Microscopy Research and Technique. DOI 10.1002/jemt.20652. ISSN: 1059-910X. IF=1.644
4. 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. - accepted for publication in Journal of Histochemistry & Cytochemistry. DOI:10.1369/jhc.2008.953018. ISSN: 0022-1554. IF=2.335

Papers and abstracts in congress proceedings:

1. Burdíková, Z., Čapek, M., Holcová, K. Kubínová, L.: The use of confocal and two photon excitation microscopy to study testate amoebae. Proceedings of PhD Students Workshop, Seč, June 2-4, 2008, Physiological Research 57: 73P-74P, 2008a. ISSN 0862-8408
2. Burdíková, Z., Kubínová, L., Čapek, M., Machač, J.: Využití konfokální a dvoufotonové mikroskopie pro studium krytének. Sborník abstraktů Mikroskopie 2008, Nové Město na Moravě, 7.-8.2. 2008, p.27, 2008b.
3. Burdíková, Z., Čapek, M., Holcová, K. Kubínová, L.: Study of testate amoeba ecology by confocal and two-photon microscopy. Abstracts, 9th Paleontological Conference, Warszawa, October 10-11, 2008, pp. 18-19, 2008c. ISBN: 978-83-61236-01-6
4. Čapek, M., Janáček, J., Kubínová, L., Smrčka, P., Hána, K.: Error assessment of volume reconstruction of biological specimens from confocal microscopy images. Proceedings of Workshop 2008, Prague, February, 2008, Volume 12, , pp. 438-439, 2008a. ISBN 978-80-01-04016-4
5. Čapek, M., Brůža, P., Janáček, J., Karen, P., Kubínová, L., Vágnerová, R., Hána, K., Smrčka, P.: 3D reconstruction of large tissue specimens using confocal microscopy data and correction of deformations by elastic registration. Proceedings of YBERC´08, Praha, June, 2008, Lékař a technika 38(2): 92-96, 2008b. ISSN 0301-5491
6. Čapek, M., Brůža, P., Kocandová, L., Janáček, J., Kubínová, L., Vagnerová, R.: Compensation and evaluation of errors of 3D reconstructions from confocal microscopic images. Proceedings of 14th European Microscopy Congress, Aachen, September 1-5, 2008, Vol. 1, Heidelberg: Springer, pp.781-782, 2008c. ISBN 978-3-540-85154-7
7. Čapek, M., Janáček, J., Kubínová, L., Smrčka, P., Hána, K.: Stanovení chyby objemové rekonstrukce biologických vzorků z konfokálních dat. Sborník abstraktů Mikroskopie 2008, Nové Město na Moravě, 7.-8.2. 2008, p.28, 2008d.

8. Čapek, M., Janáček, J., Kubínová, L., Hána, K., Smrčka, P. , Brůža, P.: 3D reconstruction of images captured by confocal microscopy from large biological specimens with rectification of deformations by elastic registration. XIII International Congress of Histochemistry and Cytochemistry Abstract Book, Gdansk, 23-27 August, 2008, Folia Histochemica et Cytobiologica 46(Suppl. 2): S131, 2008e. ISSN 0239-8508
9. Kubínová, L., Janáček, J.: Measurement of surface area of biological structures, based on 3D microscopic image data. Proceedings of 14th European Microscopy Congress, Aachen, September 1-5, 2008, Vol. 1, Heidelberg: Springer, pp.785-786, 2008. ISBN 978-3-540-85154-7
10. Vyhnal, A.: Modelling and analysis of clustering and colocalization patterns in ultrastructural immunogold labelling of cell compartments based on 3-D image data. Proceedings of 14th European Microscopy Congress, Aachen, September 1-5, 2008, Vol. 1, Heidelberg: Springer, pp.793-794, 2008. ISBN 978-3-540-85154-7
Invited lectures:
1. Kubínová, L.: Measurement of 3D image data acquired by confocal microscopy using image analysis and stereological methods. Seminar at Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, September 8, 2008 - invited lecture.
2. Kubínová, L., Janáček, J.: Estimation of geometrical characteristics of biological structures using confocal microscopy, image analysis and stereology. Shape and Size in Medicine, Biotechnology and Materials Science, Workshop of an ECMI Special Interest Group, Milano, May 27, 2008 - invited lecture.

Číslo aktivity
LK03-8
Ke kterému dílčímu cíli se aktivita vztahuje
V004 - Vývoj nových metod pro předzpracování a analýzu obrazových dat získaných konfoká...
Název (cíl)aktivity
Analysis of data acquired by confocal and two-photon microscopy using different fluorescence microscopy techniques – stage 3
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
One- and two-photon microscopic techniques available in our lab were applied to the acquisition of further data from biological material, mainly provided by the cooperating teams of the Centre. Further possibilities of two-photon fluorescence microscopy and second harmonic generation imaging for in vivo studies were checked for different biological materials (Chernyavskiy and Kubínová, 2008). We have shown that combination of different imaging modes using one- and two-photon excitation, such as 1PE reflectance, 1PE fluorescence, SHG imaging, and 2PE autofluorescence bring valuable information for studies of experimental mouse melanoma in vivo (Chernyavskiy et al., accepted to Microsc. Res. Techn., Chernyavskiy et al., 2008a,b), and for visualization of type I and type II collagen in cartilage and tendon tissues (Burdíková et al., 2008). In cooperation with Prof. Palkova's team (aim V008), we optimized imaging of yeast colonies by one-photon and especially two-photon microscopy (publication submitted to Environmental Microbiology), for details see ZP02-8. Further, we studied the correlation

between two microscopic phase-imaging modes applied to biological specimens of different optical thickness (Pelc et a., 2008). In cooperation with Doc. Svoboda's team (aim V005), we optimized FRAP measurements for their analysis of the effect of cholesterol level alterations in plasma membrane on the TRH receptor mobility (Ostašov et al., 2008a,b), see PS03-8, PS05-8.
Persons involved: Chernyavskiy, Pelc, Burdíková, Kubínová, Klepetář

Skutečné Indikátory dosažení - výsledky aktivity

In cooperation with other teams of the Centre, extensive image data were acquired by fluorescence microscopy techniques and analyzed. Improved microscopy techniques are available for all teams involved in the Centre.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity

Achieved results were presented at international congresses and published (or accepted for publication) in two peer-reviewed journals, i.e. Microscopy Research and Technique (IF=1.644), and Journal of Biomedical Optics (IF=3.084). Further, a revised version of manu submitted to Environmental Microbiology (IF = 4.929) is in preparation, see ZP02-8.

Papers in peer-reviewed journals:

1. 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. - accepted for publication in Microscopy Research and Technique. DOI 10.1002/jemt.20687. ISSN: 1059-910X. IF=1.644
2. 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=3.084

Papers and abstracts in congress proceedings:

1. Burdíková, Z., Filová, E., Rampichová, M., Bianchini, P., Čapek, M., Amler, E., Kubínová, L.: Two photon excitation microscopy and SHG imaging as a tool for visualisation of type I and type II collagen, and their use in tissue engineering. Proceedings of 14th European Microscopy Congress, Aachen, September 1-5, 2008, Vol. 3, Heidelberg: Springer, pp.199-200, 2008. ISBN 978-3-540-85154-7
2. Chernyavskiy, O., Kubínová, L.: Multi-photon excitation microscopy. Proceedings of Advances in Widefield and Confocal Fluorescence Microscopy, Ljubljana, March 13-14, 2008, pp.20-28, 2008. ISBN 978-80-7399-377-1
3. Chernyavskiy, O., Vannucci, L., Bianchini, P., Difato, F., Kubinova, L.: Non-invasive in vivo study of experimental melanoma by combination of confocal and two-photon microscopy using autofluorescence and second harmonic generation. Proceedings of ELMI, Davos, Switzerland, 27-30 May, 2008, p. 94, 2008a.
4. Chernyavskiy, O., Vannucci, L., Bianchini, P., Difato, F., Kubínová, L.: Possibilities of combination of confocal microscopy and second harmonic generation imaging for in vivo study of experimental melanoma. Proceedings of PhD Students Workshop, Seč, June 2-4, 2008, Physiological Research 57: 77P, 2008b. ISSN 0862-8408
5. Ostašov, P., Burdíková, Z., Kubínová, L., Svoboda, P.: The effect of cholesterol depletion on the thyrotropin releasing hormone receptor distribution and mobility in intact cells. Proceedings of PhD Students Workshop, Seč, June 2-4, 2008, Physiological Research 57: 82P, 2008a, ISSN 0862-8408, see PS03-8.
6. Ostašov, P., Burdíková, Z., Kubínová, L., Svoboda, P.: Detection of the Effect of Cholesterol Level Alterations in Plasma Membrane on the TRH Receptor Mobility with Fluorescence

Recovery after Photobleaching. XIII International Congress of Histochemistry and Cytochemistry Abstract Book, Gdansk, 23-27 August, 2008, Folia Histochemica et Cytobiologica 46(Suppl. 2): S105, 2008b, ISSN 0239-8508, see PS03-8.

Číslo aktivity
MH01-8
Ke kterému dílčímu cíli se aktivita vztahuje
V001 - Vývoj a aplikace technik s citlivostí jednotlivých molekul: a) kvantitativní urč...
Název (cíl)aktivity
Using FLCS as an unique and new tool for the simultanous determination of diffusion coefficients in the two opposing leaflets of biomembranes- stage 2
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
It is certainly an old dream of membrane biophysicists to simultaneously determine the lipid diffusion coefficients in both membrane leaflets. Certainly fluorescence lifetime correlation spectroscopy (FLCS) a method which was developed supported by the funding of the center has the potential in combination with the idea of lifetime tuning by quenching surfaces to reach that aim. However, for a successful application of this technique the lifetime patterns of Rhodamine, Oregon green and Bodipy head-labeled lipid analogues in both layers are necessary. All the attempts to record those “isolated” pattern were up to know unsuccessful. On the other hand the lifetime tuning approach has been used to determine the flip-flop kinetics of a series of lipid analogues, adding useful information to the issue of membrane asymmetry.
Skutečné Indikátory dosažení - výsledky aktivity
The flip-flop kinetics of a series of fluorescently labelled lipid analogues within supported lipid bilayers has been determined. Interestingly this process in SLB""s occurs on the time scale of hours which is in contraciction to considerably slower flip-flop kinteics assumed for cell membranes. It should be pointed out that the application of the lifetime tuning approach for this purpose is new.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
The flip-flop project has to be finalised in stage 3 of this project. The results will be published in journal with impact factor.

Číslo aktivity
MH02-8
Ke kterému dílčímu cíli se aktivita vztahuje
V001 - Vývoj a aplikace technik s citlivostí jednotlivých molekul: a) kvantitativní urč...
Název (cíl)aktivity
FLCS as a new tool for the characterisation of protein (peptide)-membrane interactions-stage 2: Combination with Ellipsometry, laser scanning microscopy, Z-scan fluorescence correlation spectroscopy, and fluorescence anti-bunching experiments
Zahájení aktivity

1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
We continued to characterise protein (peptide)-membrane interactions by applying complementary methods like ellipsometry, laser scanning microscopy, Z-scan fluorescence correlation spectroscopy, and fluorescence anti-bunching experiments. The protein (peptide)-membrane topics performed were: a) membrane binding of blood coagulation proteins and b) interaction of several relevant antibacterial peptides with membranes
Skutečné Indikátory dosažení - výsledky aktivity
Understanding of the mechanism of the interaction of well choosen blood coagulation proteins as well as antibacterial peptides with lipid membranes.
Experiments will be continued in 2009
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
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)

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)

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.

The results were also presented by Radek Macháň as a poster at 30th European Peptide Symposium, Helsinki, 31 August - 5 September 2008, and 4th Wroclaw-Prague Seminar on Biophysics of Lipids

Radek Macháň, Piotr Jurkiewicz, Aleš Benda, Martin Hof
"Model peptide LAH4 and its interaction with phospholipid bilayers"(2008) Journal of Peptide Science, 14(8): S176-176

Číslo aktivity
MH03-8
Ke kterému dílčímu cíli se aktivita vztahuje
V002 - Vývoj nových nosičů pro nevirální genovou terapii; monitorování osudu vzniklé ko...
Název (cíl)aktivity
FLCS as new tool for investigating the internal dynamics during DNA condensation-stage 2
Zahájení aktivity
1.1.2008
Ukončení aktivity

31.12.2008

Popis aktivity

In 2007 this new method was established for getting information on the intramolecule dynamics and dynamic equilibrium between the free and condensed form. As a results of that we for the first time revealed the Mechanism of Intermediate-sized Circular DNA Compaction Mediated by Spermine. In 2008 this method has been a) furher improved and b) applied to other condensers.

Skutečné Indikátory dosažení - výsledky aktivity

It has to be stressed that the introduction of FLCS into the field of DNA condensatiom opened totaly new possibilities for the scientific community. In 2008 we showed that this single molecule method allows on a robust way to distinguish between different compaction mechanisms. We suppose that by futher prpoagation if the FLCS technique the researchers in this field will become interested to use this unique technique, which indeed was developed and introduced by the goup Hof. This estimation may be illustrated by an invited lecture of Martin Hof at the 7th International Weber Symposium on Innovative Fluorescence Methodologies in Kauai, USA.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity

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)

Jana Humpolíčková, Aleš Benda, Martin Hof
"The compaction mechanism of an intermediate-sized DNA molecule elucidated by fluorescence lifetime correlation spectroscopy" Chemické listy, in press. (IF=0.4)

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" (2008) Journal of Fluorescence, 18(3-4, 679-684 (IF=2.6)

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, in press (IF=4.1)

The results were presented in the form of an invited lecture of Martin Hof at the 7th International Weber Symposium on Innovative Fluorescence Methodologies in Kauai, USA.

Číslo aktivity

MH04-8

Ke kterému dílčímu cíli se aktivita vztahuje

V002 - Vývoj nových nosičů pro nevirální genovou terapii; monitorování osudu vzniklé ko...

Název (cíl)aktivity

Testing of new condensing agents and DNA carriers by FCS, FLCS and solvent relaxation technique- stage 2

Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
1) In collaboration with a synthetic laboratories in France we have been characterisng the condensing efficency of newly synthesised condensers by FCS and FLCS. Based on our experience, we used Picogreen as the fluorescent label. 2)In collaboration with the Institute of Macromolecular Chemistry (CAS) polymer based DNA carriers were characterised by FCS and FLCS. 3) Liposomes based DNA carriers and other relevant lipid systems will be investigated by the solvent relaxation technique.
Skutečné Indikátory dosažení - výsledky aktivity
It can be expected that this project will lead to the development of new condesing agents and polymer or liposome based DNA carriers, which do have superior properties than those presently used.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
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)

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.

Agnieszka Olžyńska, Piotr Jurkiewicz, Martin Hof
"Properties of Mixed Cationic Membranes studied by Fluorescence Solvent Relaxation" Journal of Fluorescence, 2008, 18(5), 925-928 (IF=2.6)

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, 2008, 112(16), 3506-3514 (IF=3.0)

Číslo aktivity
MH05-8
Ke kterému dílčímu cíli se aktivita vztahuje
V001 - Vývoj a aplikace technik s citlivostí jednotlivých molekul: a) kvantitativní urč...
Název (cíl)aktivity
Application of the newly developed single molecule techniques in other fields.
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008

Popis aktivity
1) Selfassembly of porphyrins with calix[4]arenes has been studied by fluorescence lifetime imaging. 2) Single silicon nanocrystals wre studied by fluorescence flucutaion analysis.
Skutečné Indikátory dosažení - výsledky aktivity
The results further demonstrate the versatility of the single molecule fluorescence approaches developed in the group Hof.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
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)
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, Roč. 25, -, s. 299-307.(IF=2.0)

Kubát, Pavel Lang, Kamil Lhoták, P. Janda, Pavel Sýkora, Jan Matějíček, P. Hof, Martin Procházka, K. Zelinger, Zdeněk. Porphyrin/calixarene self-assemblies in aqueous solution. Journal of Photochemistry and Photobiology. A - Chemistry Section, 2008, Roč. 198, č. 1, s. 18-25.(IF=1.9)

Číslo aktivity
MH06-8
Ke kterému dílčímu cíli se aktivita vztahuje
V001 - Vývoj a aplikace technik s citlivostí jednotlivých molekul: a) kvantitativní urč...
Název (cíl)aktivity
Establishing Homo-Energytransfer as a tool for determination of co-localisation in membranes of living cells and model membranes
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
Homo-Energytransfer leads to a decrease in the fluorescence anisotropy. Thus the formation of protein or lipid dimers can be monitored by using a single labelling approach. Ezperiments were performed in model systems, like micelles, as well as in living cells. However, experiments on living cells appear to be hampered by technical set up problems at the microscope. In 2009 we will continue these efforts.
Skutečné Indikátory dosažení - výsledky aktivity
Establishing a new in vivo imaging tool, which is relevant for V005-V008.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Mainly, in form of publication in high impact factor journal

Číslo aktivity
MH07-8
Ke kterému dílčímu cíli se aktivita vztahuje
V001 - Vývoj a aplikace technik s citlivostí jednotlivých molekul: a) kvantitativní urč...

Název (cíl)aktivity
Application of advanced fluorescence techniques in the elucidation of the structure function relationship of dehalogenase proteins
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
Haloalkane dehalogenases catalyze hydrolytic cleavage of carbon-halogen bonds in the halogenated aliphatic compounds, producing a corresponding alcohol, a halide ion and a proton. Therefore those proteins are in the focus of interest in terms of environmental biochemistry as well as for the development of biosensors. In order to optimise those proteins an understanding of the structure function relationship of the proteins is necessary. In 2008 we applied advanced fluorescence spectroscopy to this end.
Skutečné Indikátory dosažení - výsledky aktivity
The tunnel mouths are evolutionally the most variable regions in the structures of haloalkane dehalogenases originating from different bacterial species suggesting their importance for adaptation of enzymes to various substrates. We decided to monitor the dynamics of this particular region by means of time resolved fluorescence spectroscopy. To label the enzyme specifically, we -in close collaboration with Prof. Damborsky from the university BRNO) adapted a novel procedure that utilizes a coumarin dye containing a halide-hydrocarbon linker, which serves as a substrate for enzymatic reaction. The procedure leads to a coumarin dye covalently attached and specifically located in the tunnel mouth of the enzyme. In this manner, we stained two haloalkane dehalogenase mutants, DbjA-H280F and DhaA-H272F. The measurements of time-resolved fluorescence anisotropy, acrylamide quenching and time resolved emission spectra reveal differences in the polarity, accessibility and mobility of the dye and its microenvironment for both of the mutants. The obtained experimental data are consistent with the results obtained by molecular dynamics calculations and correlate with the anatomy of the tunnel mouths, which were proposed to have a strong impact on the catalytic activity and specificity of the examined mutants. Interestingly, the kinetics of the recorded time-dependent Stokes shift is unusually slow it occurs on the nanosecond time-scale, suggesting that the protein dynamics is extremely slowed down at the region involved in the exchange of ligands between the active site cavity and bulk solvent.
Theese result appear soon in a very prestigios Journal: Journal of the American Chemical Society

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Jesenska, A Sykora, J Olzynska,A Bresovsky, J Zdrahal, Z Damborsky, J Hof, M Journal of the American Chemical Society, in press. (IF=7.9)

Číslo aktivity
MH08-8
Ke kterému dílčímu cíli se aktivita vztahuje
V001 - Vývoj a aplikace technik s citlivostí jednotlivých molekul: a) kvantitativní urč...
Název (cíl)aktivity
4th Wroclaw-Prague Seminar on Biophysics of Lipids

Zahájení aktivity
23.10.2008
Ukončení aktivity
25.10.2008
Popis aktivity
M Hof was the co-organiser of the 4th Wroclaw-Prague Seminar on Biophysics of Lipids, organized in Wroclaw, 23.10.2008 - 25.10.2008.
Skutečné Indikátory dosažení - výsledky aktivity
Conference with focus on Advanced fluorescence in biomembrane research, 60 participants including 45 from abroad.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Conference proceeding without ISBN number

Číslo aktivity
PS01-8
Ke kterému dílčímu cíli se aktivita vztahuje
V005 - Analýza molekulárního mechanismu desensibilizace hormonální akce, určení změn v ...
Název (cíl)aktivity
Determination of detergent effect on steady-state anisotropy of DPH in plasma membrane preparations - stage 2: The effect of detergents on trimeric G-protein activity in PM from rat brain cortex correlation with studies of DPH and Laurdan fluorescence
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
The biophysical state of hydro-phobic interior of plasma membrane (PM) is an important determinant of GPCR action. The conformational state of receptor molecule as well as interaction with G-protein(s) is regulated by saturation/ un-saturation state of fatty acids in membrane lipids, by varying cholesterol amounts and by other factors such as concentrations of monovalent and/or divalent cations, ionic strength and specific amphiphilic molecules like 4-hydroxystearate. Furthermore, studies of PM solubilization and/or resistance to various detergents indicate an agonist-specific conformation, which is sensitive to rious effect of high detergent concentrations. In this work, we have used the hydro-phobic fluorescent probe diphenylhexatriene (DPH) as an “indicator” of the membrane state representing both structural and dynamic parameters. The effect of non-ionic detergents on baclofen (GABAB-R agonist)-stimulated G-protein activity was measured as [35S]GTPγS binding assay. Our experiments were performed with purified plasma membrane fraction (PM) isolated from rat brain cortex. The membrane-water interface was characterized by generalized polarization of Laurdan fluorescence (GP). Steady-state anisotropy measurements indicated a marked detergent effect. When increasing detergent concentration, the decrease of fluorescence anisotropy r (DPH) was clearly detected. The same effect was elicited by decrease of temperature – equivalent to temperature shift-down of about 25°C. At high detergent concentrations, fluorescence anisotropy of membranes plus detergent was close to the highly depolarized signal of DPH in detergent solution alone.
Skutečné Indikátory dosažení - výsledky aktivity

In brain membranes, detergent effect on r (DPH) could be correlated with increase of GABAB-receptor stimulated G-protein activity. The effect was clearly biphasic - decrease in activity was followed by activation/maximum and finally, at high concentrations, drastic inhibition of G-protein activity was noticed. Contrarily, specific radioligand binding to GABAB-receptor was inhibited in the whole range of detergent concentrations step by step, i.e. it was strictly monophasic. The magnitude of the both detergent effects was decreased with the same order of potency: Brij58 > Triton X-100 > Digitonin.
The same order was found when comparing detergents ability to alter fluorescence anisotropy of the membrane probe 1, 6-diphenyl-1,3,5-hexatriene (r DPH) incorporated into the hydro-phobic PM interior. Decrease of r DPH, in the order of Brij58 > Triton X-100 > Digitonin, was reflected as decrease of the S-order parameter and rotation correlation time Phi paralleled by an increase of diffusion wobbling constant Dw (analysis by time-resolved fluorescence according to "vobling-in-cone" model). As before, the effect of detergents on GP parameters of Laurdan fluorescence proceeded with the order: Brij58 > Triton X-100 > Digitonin.
Our data indicate that an optimum perturbation of the native PM structure is advantageous for the functional coupling between receptor and its cognate G-protein. Both hydro-phobic membrane phase and the water-membrane interface participate in this process.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity

Sykora, J., Bourova, L., Hof, M. and Svoboda, P. (2008) 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.BBA Biomembranes, 2008 Nov 21. [Epub ahead of print], in print (IF=3.6)

Číslo aktivity

PS02-8

Ke kterému dílčímu cíli se aktivita vztahuje

V005 - Analýza molekulárního mechanismu desensibilizace hormonální akce, určení změn v ...

Název (cíl)aktivity

Isolation of plasma membrane compartments from rat brain cortex detection of agonist-stimulated G-protein activity

Zahájení aktivity

1.1.2008

Ukončení aktivity

31.12.2008

Popis aktivity

New method for isolation of purified PM from rat brain cortex was developed. This method is based on mild homogenization and centrifugation in two types of density gradients. First, Percoll is used for separation of plasma membrane and mitochondrial fractions, subsequently, PM are sub-fractionated floatation in discontinuous sucrose gradient. Percoll-purified PM had been used for determination of trimeric G-protein activity.

Skutečné Indikátory dosažení - výsledky aktivity

We have found that determination of G-protein activity in rat brain cortex membranes requires specific methodological conditions when compared with HEK293 cell lines expressing various types of GPCR (DOR, TRH-R). When using GTPgammaS binding assay for determination of G-protein activity in brain tissue, relatively high concentrations of GDP had to be included in

binding assay medium and protein concentration in reaction mix had to be decreased bellow 20 ug of PM protein per sample. An optimum range of GDP concentrations for detection of baclofen-stimulated GTPgammaS binding was at 20–30 uM GDP. Optimum protein concentration was 10 ug or less.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity

Bourova, L., Stöhr, J., Lisy, V., Rudajev, V., Novotny, J. and Svoboda, P. (2008) Isolation of plasma membrane compartments from rat brain cortex detection of agonist-stimulated G protein activity. Medical Science Monitor (MCM), accepted (IF=1.6)

Lenka Bourova, Jiri Stohr, Vaclav Lisy, Vladimir Rudajev, Jiri Novotny and Petr Svoboda (2008) Subcellular fractionation of rat brain cortex detection of agonist-stimulated G protein activity. Key-Stone Meeting "G protein-coupled Receptors: New Insights in Functional Regulation and Clinical Application", Killarney, Kerry, Ireland, May 18-23, 2008

Číslo aktivity

PS03-8

Ke kterému dílčímu cíli se aktivita vztahuje

V005 - Analýza molekulárního mechanismu desensibilizace hormonální akce, určení změn v ...

Název (cíl)aktivity

Time-resolved FRET analysis of cell membrane proteins of interest - stage 2 has been postponed in favour of FRAP

Zahájení aktivity

1.1.2008

Ukončení aktivity

31.12.2008

Popis aktivity

1) After optimization of method of preparation of Percoll purified PM, due to differences in cells expressing TRH-R-eGFP fusion proteins to other cells, the membranes were prepared. For this reason only a few test FLIM images on living cells were taken to prove that eGFP within TRH-R-eGFP fussion protein is usable for futher experiments and prone to lifetime changes. We also dicovered undocumented mutations in our plasmids which effectively preveted expression of fussion proteins they were carrying. After careful consideration we decided not to continue our program oriented to FRET analysis of G alpha subunit oligomerisation using donor-acceptor pairs like CFP-YFP, eGFP-YFP or YFP-DsRed. All these fluorescent analogs of GFP are too bulky molecules (about 28 kDa) when compared with G alpha subunits (about 40 kDa). It is reasonable to assume that ion of this large molecule into the G alpha subunit will alter the native conformation of G alpha to an extent that it will be unable to respond to hormonal stimulation mediated via activated receptor. The chance that the agonist-induced conformational change of receptor molecule will be transmitted further down-stream, i.e. to Gα-eGFP or Gα-CFP does not appear to be likely from the present scope of our experimentation at least. 2) We analysed changes in distribution and diffusion of fluorescently labelled TRH receptor caused by cholesterol depletion in living cells. In collaboration with Burdikova from Dr. Kubinova team we examined those changes using confocal microscopy and fluorescence recovery after photobleaching (FRAP).

Skutečné Indikátory dosažení - výsledky aktivity

1) We finished equipment of special tissue culture room (the laminar flow box, CO2 incubator, incubation bath), which is necessary for work with tissue-cultures in Heyrovsky Institute. Although we made some initial tests for FLIM the obtained data are not sufficient to determine meaningful values of lifetime of eGFP contained within TRH-R-eGFP molecule. More experiments will be needed to optimize protocol to ensure that obtained values are not affected by our setup.
2) Cholesterol depletion have not changed TRH receptor distribution, however caused changes in its diffusion parameters. From data aquired from FRAP experiments with different setups we conclude that under control conditions is receptor part of larger complex, which is disrupted by cholesterol depletion.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
P. Ostasov, Z. Burdikova, L. Kubinova, P. Svoboda (2008) The effect of cholesterol depletion on the thyrotropin releasing hormone receptor distribution and mobility in intact cells. Annual Meeting of Institute of Physiology AS CR, Seč, CZ, June 2008

Pavel Ostasov, Zdena Burdíková, Lucie Kubínová and Petr Svoboda (2008) Detection of the effects of cholesterol level alternations in plasma membrane on TRH receptor mobility with fluorescence recovery after photobleaching. "13th Congress of the International Federation of Societies for Histochemistry and Cytochemistry", Gdansk, Poland, August 24-28
Pavel Ostasov, Jana Brejchova, Jiri Novotny and Petr Svoboda (2008) Distribution of TRH receptor in plasma (cell) membrane as revealed by confocal laser scanning microscopy of living cells expressing thyrotropin-releasing hormone receptor-GFP fusion protein (TRH-R-GFP). Key-Stone Meeting "G protein-coupled Receptors: New Insights in Functional Regulation and Clinical Application", Killarney, Kerry, Ireland, May 18-23, 2008

Číslo aktivity
PS04-8
Ke kterému dílčímu cíli se aktivita vztahuje
V005 - Analýza molekulárního mechanismu desensibilizace hormonální akce, určení změn v ...
Název (cíl)aktivity
The role of plasma membrane integrity in mechanism of hormone action and desensitization: Time-course of agonist-induced solubilization of trimeric Gq/G11 alpha proteins resolved by two-dimensional electrophoresis and changes in protein composition of PM
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
1) We have studied effect of TRH receptor activation on redistribution of G protein between plasma membrane (PM) and cytosol (HEK293 cells). In this study, we have used 2D electrophoresis for more defined resolution of Galpha subunits of Gq/G11 family and followed the time course of solubilisation effect. 2) We aimed to detect changes in plasma membrane protein composition after prolonged stimulation (16 hours) with thyrotropin-releasing hormone (TRH) using 2D electrophoresis and Sypro Ruby staining.

Skutečné Indikátory dosažení - výsledky aktivity
1) Prolonged agonist stimulation results in specific transfer of activated Galpha subunits of Gq/G11 alpha family from particulate membrane fraction to soluble (cytosol) cell fraction isolated as 250,000 x g supernatant. The small signal of soluble G proteins was detected already in control, hormone-unexposed cells. Hormone stimulation resulted in slow but continuous increase of both intensity and number of immuno-reactive signals/spots of these G proteins (10, 30, 60, 120 and 240 min). At longer times of agonist exposure (>2 hours), marked increase of Gq/G11 alpha proteins was detected. The maximal level of soluble Gq/G11 alpha proteins was reached after 16 hours of continuous agonist exposure. At this time interval, eight individual immuno-reactive signals of Gq/G11 alpha proteins could have been resolved. Solubilisation of this class of Galpha proteins was thus observed after prolonged agonist stimulation only, induced by ultra high concentration of hormone and in cells expressing large number of GPCRs. Our data therefore indicate rather tight/persisting binding of Gq/G11 alpha proteins to the membrane.
2) We used an improved sample solubilization method and purification of plasma membrane proteins on Percoll-gradient. This setup enabled a more sensitive and precise detection of protein composition of the plasma membrane and thus we could detect a clear alteration of an overall protein composition in isolated plasma membranes purified from cells exposed to TRH. Disapperance of 3 proteins and decrease in the amount of 18 proteins was observed in samples isolated from hormone treated cells. This occurred in parallel with specific decrease in the amount of Gq/G11 alpha proteins, which was detected by an immunoblot and decrease of functional activity of these G proteins measured by agonist-stimulated [35S]GTPγS binding assay.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
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)

Zdenka Drastichova, Lenka Bourova and Petr Svoboda (2008) Changes in protein composition of plasma membranes from HEK293 cells expressing high levels of G11alpha protein after prolonged treatment with thyrotropin-releasing hormone. Key-Stone Meeting “G protein-coupled Receptors: New Insights in Functional Regulation and Clinical Application”, Killarney, Kerry, Ireland, May 18-23, 2008

Zdenka Drastichová, Lenka Bouřová, Jiří Novotný and Petr Svoboda (2008) 2-D electrophoretic resolution of PercollR-purified plasma membranes isolated from HEK293 cells expressing thyrotropin-releasing hormone receptor and G11alpha protein comparison of control and “down-regulated” cells. Annual Meeting of Institute of Physiology, AS CR, Seč, CZ, June 2008

Číslo aktivity
PS05-8
Ke kterému dílčímu cíli se aktivita vztahuje
V005 - Analýza molekulárního mechanismu desensibilizace hormonální akce, určení změn v ...
Název (cíl)aktivity
Disruption of the plasma membrane integrity by cholesterol depletion alter TRH receptor-mediated signal transduction via Gq/G11 alpha proteins

Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
1) We used confocal laser scanning microscopy (CLSM) to detect distribution of TRH-R-GFP and its changes in HEK 293 cells expressing this receptor. Cells were treated with beta-cyclodextrin (beta-CD) in order to deplete cholesterol from the plasma membrane and alter its integrity. 2) We studied the process of agonist-induced desensitization of Ca2+ responses in HEK293 cells transfected to express high levels of thyrotropin-releasing hormone (TRH) receptors (clone E2) or TRH receptors along with G11 alpha protein (clone E2M11). Both these cell lines also expressed endogenous angiotensin II (ANG II) receptors.
Skutečné Indikátory dosažení - výsledky aktivity
1) Under control conditions distribution TRH-R appeared mostly homogenous with exception of areas where two cells attach to each other. These areas showed much higher intensity of fluorescence, similarly as the cell processes and their bases. Neither recpetor distribution nor overall cell morphology was affected by cholesterol depletion. Althougt pretreatement of cells with bata-CD did not cause any visible changes in the distribution of the fluorescence signal, this intervention slowed down the process of the receptor internalisation.
2) The treatment of E2 cells with TRH or ANG II led to significant desensitization of the Ca2+ response to subsequent addition of either hormone. The response was not desensitized in E2M11 cells expressing high levels of G11 alpha. In addition, stimulation of both cell lines with TRH elicited a clear heterologous desensitization to subsequent stimulation with ANG II. Moreover, cholesterol depletion prolonged the lag phase and reduced the maximal TRH-induced Ca2+ response. Depletion of cholesterol wiped out the Ca2+ response to 1 nM TRH, but the Ca2+ response to 1 uM TRH was well preserved in E2 cells. Furthermore, cholesterol depletion allowed a partial re-sensitization of E2 cells stimulated by 1 uM TRH to a second challenge with the same hormone.

These observations indicate that intact structure of the plasma membrane is important for proper regulation of TRH-R and Gq/11 alpha-mediated transmembrane signalling.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Pavel Ostasov, Jana Brejchova, Jiri Novotny and Petr Svoboda (2008) Distribution of TRH receptor in plasma (cell) membrane as revealed by confocal laser scanning microscopy of living cells expressing thyrotropin-releasing hormone receptor-GFP fusion protein (TRH-R-GFP). Key-Stone Meeting “G protein-coupled Receptors: New Insights in Functional Regulation and Clinical Application”, Killarney, Kerry, Ireland, May 18-23, 2008
Jiri Novotny, Pavel Ostasov, Jan Krusek and Petr Svoboda (2008) Desensitization of Ca2+ responses to thyrotropin-releasing hormone and angiotensin II: the role of plasma membrane integrity and temperature. Key-Stone Meeting “G protein-coupled Receptors: New Insights in Functional Regulation and Clinical Application”, Killarney, Kerry, Ireland, May 18-23, 2008

Číslo aktivity
VM01-8
Ke kterému dílčímu cíli se aktivita vztahuje
V003 - Vývoj modelových systémů biologických membrán založených na polarizovatelném kap...
Název (cíl)aktivity
The behaviour of a phospholipid layer at the water/1,2-dichloroethane interface within a range of interfacial potential differences below the zero-charge potential difference.
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
The behaviour of L-α-lecithin (DPPC) was studied at the water/1,2-dichloroethane interface at negative potential differences using surface tension and voltammetric measurements. The aim of this study is to explain the surface pressure maximum of adsorbed DPPC occuring in acidic media close to the zero charge potential difference. Formation and stability of a mixed phospholipid layer of DPPC and DPPA (phosphatidic acid) at a polarized liquid/liquid interface.
Skutečné Indikátory dosažení - výsledky aktivity
The general five-step model, established in Part I and II of this series, has been confirmed. The surface pressure of DPPC adsorbed at the interface exhibits a maximum in acidic media close to the zero-charge potential difference, Ezc, of the base electrolytes. It is demonstrated that, in contrast to the potentials higher than Epzc, where the change in the surface pressure is due to desorption of the protonated form of DPPC, the observed change in the surface pressure at interfacial potential differences below Ezc can be explained by alterations in the concentrations of the adsorbed zwitter-ionic and protonated forms of DPPC governed by the general acid-base equilibrium.
The mixed adsorbed layer of DPPC and DPPA enhanced the blocking effect of potassium ion transfer. The electrochemical stability of the mixed barrier layer was maintained at pH region lower than 4. When pH was higher than 4, the blocking effect disappeared.The results are discussed in relation to the complex formation of phospholipids with ions and to their protonation.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
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)
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)

Číslo aktivity
ZP01-8
Ke kterému dílčímu cíli se aktivita vztahuje
V008 - Nové poznatky a biologické implikace o a) přítomnosti a interakci vybraných tran...
Název (cíl)aktivity
FRET analysis of possible interaction of Ato-FP proteins - stage 2
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
First FRET experiments revealed problems of rapid bleaching and relatively low stability of YFP-CFP pairs. Therefore new strain of S.cerevisiae strains containing S.cerevisiae codone-optimised enhanced fluorescent variants Venus-CFP fused with individual Ato proteins were prepared and used for FLIM/FRET experiments.
Skutečné Indikátory dosažení - výsledky aktivity
10 new strains containing different combinations of Ato-Venus and Ato-CFP fluorescent Ato proteins were prepared and verified. The method of fluorescence lifetime imaging (FLIM) was elaborated together with partner 1, with the aim to identify FRET interaction between the pairs of Ato-fluorescent protein. We found a shift of approximately 0.2 ns to be typical shortening of the donor fluorescence lifetime due to the FRET interaction between Ato1p and Ato2p. The interaction was confirmed by two convenient FRET pairs – eGFP/dsRed-tdimer and eCFP/Venus. We did not find marked interaction for the rest of combinations of Ato proteins (i.e. Ato1p and Ato3p and Ato2p and Ato3p).
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Obtained results indicate FRET interaction between Ato1p and Ato1p proteins specificaly inside the yeast plasma membrane. On the other hand, there is no interaction between Ato1p and Ato3p or between Ato2p and Ato3p. The results were confirmed by two different fluorescent pairs eGFP/dsRed-tdimer and eCFP/Venus. The data are completed and prepared to be published.

Číslo aktivity
ZP02-8
Ke kterému dílčímu cíli se aktivita vztahuje
V008 - Nové poznatky a biologické implikace o a) přítomnosti a interakci vybraných tran...
Název (cíl)aktivity
Two-photon microscopy analysis of profile of Ato1p-GFP production in colonies of parental strain and in colonies of ed mutants - stage 2
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
The analyses of production of Ato1p-GFP in microcolonies of parental strain BY4742 was suplemented with the analysis of localisation of Ato1p-dsRed variant. It confirmed the Ato1p-

GFP production pattern. Production of Ato1p-GFP in strains of mutants (sok1, sod2, sod1 and ctt1) was compared with that of the parental strain.

Skutečné Indikátory dosažení - výsledky aktivity

Viewing the colonies from different angles allowed us to reconstruct a three-dimensional profile of the cells producing ammonium exporter Ato1p within developing microcolonies growing either as individuals or within a group of microcolonies. We show that neighbouring microcolonies coordinate production of Ato1p-GFP during the time, however, they keep to some extent their individuality even when tightly joined. Ato1p itself appears synchronously in cells, which do not originate from one ancestor, but have specific position within the colony.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity

The obtained results were prepared and submitted for the publication for Environmental Microbiology. At present, we are designing additional experiments asked by one of the referees.
Váchová,L., Chernyavskiy, O., Strachotová, D., Bianchini, P., Burdíková, Z., Ferčíková, F., Kubínová, L., Palková, Z. Architecture of developing multicellular yeast colony: Spatio-temporal expression of Ato1p ammonium exporter.
Environmental Microbiology (IF = 4.929): revised version in preparation.

Číslo aktivity

ZP03-8

Ke kterému dílčímu cíli se aktivita vztahuje

V008 - Nové poznatky a biologické implikace o a) přítomnosti a interakci vybraných tran...

Název (cíl)aktivity

Localisation of Ato-FP transporters to either RMC-P or RMC-C compartments of plasma membrane.

Zahájení aktivity

1.1.2008

Ukončení aktivity

31.1.2009

Popis aktivity

Raft distribution of Ato-FP transporters and their co-localisation with RMC-P and RMC-C compartments of plasma membrane, respectively, will be analyzed. For this purpose, new strains containing protein markers for RMC-P and RMC-C kompartments (e.g. Pma1p, Can1p etc.) together with Ato-FP will be prepared and used for co-localiation experiments.

Skutečné Indikátory dosažení - výsledky aktivity

Until now, the strain containing plasma membrane H+ ATPase Pma1p (marker of RMC-P)fused with RFP was constructed and verified and used for preparation of the strain containing also Ato1p labelled with GFP. The construction of this strain was more complicated that supposed because of problems with viability of colonies of strains containing Pma1p-dsRed fusions.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity

New strains containing Pma1p-RFP and Ato1p-GFP, which will be used for co-localisation studies.

Číslo aktivity
ZP04-8
Ke kterému dílčímu cíli se aktivita vztahuje
V008 - Nové poznatky a biologické implikace o a) přítomnosti a interakci vybraných tran...
Název (cíl)aktivity
Analyses of the effect of pH and other factors on productions of Ato proteins in situ within monocolonies - stage 1
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2010
Popis aktivity
Together with the partner 2, the first experiments were performed with the aim to check the effect of ammonia on cells of colonies in situ.
Skutečné Indikátory dosažení - výsledky aktivity
Under conditions of increased ammonia (within the covering agarose layear) Ato1p-GFP production enhances in surface colony layers.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Some of the results will become part of the publication.

Číslo aktivity
ZP05-8
Ke kterému dílčímu cíli se aktivita vztahuje
V008 - Nové poznatky a biologické implikace o a) přítomnosti a interakci vybraných tran...
Název (cíl)aktivity
Development of codon-optimized pH- and redox-sensitive GFP probes for in vivo organellar measurements - stage 1
Zahájení aktivity
1.5.2008
Ukončení aktivity
31.12.2009
Popis aktivity
The study of ammonia-induced metabolic changes such as oxidative phosphorylation decrease, glyoxylate cycle activation or amino acid redistribution calls for a non-invasive method for in vivo monitoring of pH and redox potential at the subcellular level. For this purpose optimized pH- and redox-sensitive ratiometric GFP mutants will be prepared and targeted to vacuoles, cytosol, mitochondria and peroxisomes.
Skutečné Indikátory dosažení - výsledky aktivity
Yeast codon-optimized version of wild type GFP for site-directed mutagenesis was chosen in order to match the acidity constant (pKa) or redox potential (E'0) of each GFP variant with that of the organelles of interest. The protocol of site-directed mutagenesis of GFP was optimised. In parallel, optimized protocol for in vivo subcellular GFP imaging and pH titration was developed. Combining spectroscopy analysis, fluorescence lifetime imaging (FLIM), confocal and two-photon microscopy the permeabilization efficiency of several ionophores and titration buffers on cells expressing Ato1-GFP fusion proteins was assessed.

Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Optimised protocol of site-directed mutagenesis of GFP. Optimised protocol for in vivo subcellular GFP imaging and pH titration.

Číslo aktivity
ZP06-8
Ke kterému dílčímu cíli se aktivita vztahuje
V008 - Nové poznatky a biologické implikace o a) přítomnosti a interakci vybraných tran...
Název (cíl)aktivity
Monitoring of production of cell wall protein Flo11p-GFP in microcolonies
Zahájení aktivity
1.1.2008
Ukončení aktivity
31.12.2008
Popis aktivity
Following the previous results obtained, we monitored the presence of Flo11p-GFP within yeast microcolonies in more detail.
Skutečné Indikátory dosažení - výsledky aktivity
We showed (among others) that the second dimorphic transition, which occurs when smooth microcolonies start to form aerial fluffy structure, is dependent on the function of Flo11p. This protein relocalises from bud neck of cell clusters to the bud tip of elongated haploid cells and is indispensable for elongation of clustered cells and their transition to pseudohyphal growth essential for the formation of aerial guts.
Skutečné prostředky ověření - forma zpracování a předání výsledku aktivity
Obtained data (together with some additional results of the centre LC531) became part of the manu, which was submitted.
Vopálenská I., Šťovíček V., Janderová B., Váchová L., Palková Z.: Role of distinct dimorphic transitions in territory colonizing and formation of S. cerevisiae colony architecture, PLOS Pathogens: submitted