РЕЗЮМЕТА НА НАУЧНИТЕ ПУБЛИКАЦИИ НА АНГЛИЙСКИ ЕЗИК

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1.[B4] Vitkova, V., **Hazarosova, R.**, Antonova, K., Mitkova, D., Yordanova, V., Momchilova, A., Staneva, G., Resveratrol Stiffens 1-palmitoyl-2-oleoyl-snglycero-3-phosphocholine Bilayers, *Lecture Notes in Networks and Systems*, 1st International Symposium on Bioinformatics and Biomedicine, BioInfoMed 2020, Burgas, 374 LNNS, 363-371, 2022; (SJR_{2022}=0.150, Q4)
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https://link.springer.com/chapter/10.1007/978-3-030-96638-6_38

Abstract: In response to exogenous stress some plant species synthesize the phytoalexin resveratrol (3, 5, 4′-trihydroxy-trans-stilbene). Found in the skins and seeds of red grapes, red wines, peanuts and other nutrients this polyphenolic compound has been recognized as beneficial in the prevention of oxidative damage in the human organism. The mechanism by which resveratrol exerts its pleiotropic effects is still unclear. Here we study its influence on the structure, mechanics and electrical properties of biomimetic lipid systems composed of the monounsaturated lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). The degree of hydration of lipid molecules in the bilayers is assessed by means of Laurdan fluorescence spectroscopy of large unilamellar vesicles. In POPC vesicles, we report enhanced lipid ordering at all concentrations of the polyphenol studied. The increased degree of lipid order in the POPC-containing matrix supports the hypothesis that resveratrol can be considered as a “filler”, located parallel to the lipid molecules. Thermal shape fluctuation analysis of nearly spherical giant unilamellar vesicles is applied to probe the membrane mechanics in the presence of resveratrol. Higher bending elasticity modulus of POPC bilayers is measured at increasing the polyphenol concentration. At 200 μmol/L of resveratrol the membrane bending rigidity is reported to increase by nearly 20% compared to its value in bidistilled water. This

finding is coherent with the results from fluorescence spectroscopy testifying to the denser packing of POPC molecules induced by resveratrol. The reported results provide knowledge about the phytoalexin's effect on the structural organization of membrane lipids as well as on the bilayer mechanical properties. Revealing the molecular basis of resveratrol-membrane interactions helps developing future applications of the polyphenol in medicinal chemistry and pharmacology.

2.[B4] Kostadinova, A., Staneva, G., Topouzova, T., Moyankova, D., Yordanova, V., Veleva, R., Nikolova, B., Momchilova, A., Djilianov, D., **Hazarosova, R.**, Myconoside Affects the Viability of Polarized Epithelial MDCKII Cell Line by Interacting with the Plasma Membrane and the Apical Junctional Complexes, *Separations*, 2022, 9(9), 239; (IF_{2022}=3.344, Q3)
DOI: 10.3390/separations9090239;
https://www.mdpi.com/2297-8739/9/9/239

Abstract: The phenyl glycoside myconoside, extracted from Balkan endemic Haberlea rhodopensis, has a positive effect on human health, but the exact molecular mechanism of its action is still unknown. The cell membrane and its associated junctional complex are the first targets of exogenous compound action. We aimed to study the effect of myconoside on membrane organization and cytoskeleton components involved in the maintenance of cell polarity in the MDCKII cell line. By fluorescent spectroscopy and microscopy, we found that at low concentrations, myconoside increases the cell viability by enhancing membrane lipid order and adherent junctions. The opposite effect is observed in high myconoside doses. We hypothesized that the cell morphological and physicochemical changes of the analyzed cell compartments are directly related to cell viability and cell apical-basal polarity. Our finding contributes to a better understanding of the beneficial application of phytochemical myconoside in pharmacology and medicine.

3.[B4] Kostadinova, A., **Hazarosova, R.**, Topouzova-Hristova, T., Moyankova, D., Yordanova, V., Veleva, R., Nikolova, B., Momchilova, A., Djilianov, D., Staneva, G., Myconoside Interacts with the Plasma Membranes and the Actin Cytoskeleton and Provokes Cytotoxicity in Human Lung Adenocarcinoma A549 Cells, *Journal of Bioenergetics and Biomembranes*, 2022, 54(1), 31–43; (IF_{2022}=3.853, Q2)
PMID: 34988784; DOI: 10.1007/s10863-021-09928-x;
https://pubmed.ncbi.nlm.nih.gov/34988784/

Abstract: Studies have been carried out on the effects of the phenyl glycoside myconoside, extracted from the relict, Balkan endemic resurrection plant Haberlea rhodopensis on the plasma membrane structural organization and the actin cytoskeleton. Because the plasma membrane is the first target of exogenous bioactive compounds, we focused our attention on the influence of myconoside on the membrane lipid order and actin cytoskeleton in human lung adenocarcinoma A549 cells, using fluorescent spectroscopy and microscopy techniques. We found that low myconoside concentration (5 μg/ml) did not change cell viability but was

able to increase plasma membrane lipid order of the treated cells. Higher myconoside concentration (20 μg/ml) inhibited cell viability by decreasing plasma membrane lipid order and impairing actin cytoskeleton. We hypothesize that the observed changes in the plasma membrane structural organization and the actin cytoskeleton are functionally connected to cell viability. Biomimetic membranes were used to demonstrate that myconoside is able to reorganize the membrane lipids by changing the fraction of sphingomyelin-cholesterol enriched domains. Thus, we propose a putative mechanism of action of myconoside on A549 cells plasma membrane lipids as well as on actin filaments in order to explain its cytotoxic effect at high myconoside concentration.

4.[B4] Momchilova, A., Pankov, R., Staneva, G., Pankov, S., Krastev, P., Vassileva, E., **Hazarosova, R.**, Krastev, N., Robev, B., Nikolova, B., Pinkas, A., Resveratrol Affects Sphingolipid Metabolism in A549 Lung Adenocarcinoma Cells, *International Journal of Molecular Sciences*, 2022, 23(18), 10870; (IF_{2022}=5.6, Q1)
PMID: 36142801; PMCID: PMC9505893; DOI: 10.3390/ijms231810870;
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9505893/

Abstract: Resveratrol is a naturally occurring polyphenol which has various beneficial effects, such as anti-inflammatory, anti-tumor, anti-aging, antioxidant, and neuroprotective effects, among others. The anti-cancer activity of resveratrol has been related to alterations in sphingolipid metabolism. We analyzed the effect of resveratrol on the enzymes responsible for accumulation of the two sphingolipids with highest functional activity—apoptosis promoting ceramide (CER) and proliferation-stimulating sphingosine-1-phosphate (S1P)—in human lung adenocarcinoma A549 cells. Resveratrol treatment induced an increase in CER and sphingosine (SPH) and a decrease in sphingomyelin (SM) and S1P. Our results showed that the most common mode of CER accumulation, through sphingomyelinase induced hydrolysis of SM, was not responsible for a CER increase despite the reduction in SM in A549 plasma membranes. However, both the activity and the expression of CER synthase 6 were upregulated in resveratrol-treated cells, implying that CER was accumulated as a result of stimulated de novo synthesis. Furthermore, the enzyme responsible for CER hydrolysis, alkaline ceramidase, was not altered, suggesting that it was not related to changes in the CER level. The enzyme maintaining the balance between apoptosis and proliferation, sphingosine kinase 1 (SK1), was downregulated, and its expression was reduced, resulting in a decrease in S1P levels in resveratrol-treated lung adenocarcinoma cells. In addition, incubation of resveratrol-treated A549 cells with the SK1 inhibitors DMS and fingolimod additionally downregulated SK1 without affecting its expression. The present studies provide information concerning the biochemical processes underlying the influence of resveratrol on sphingolipid metabolism in A549 lung cancer cells and reveal possibilities for combined use of polyphenols with specific anti-proliferative agents that could serve as the basis for the development of complex therapeutic strategies.

5.[B4] Hazarosova, R., Momchilova, A., Vitkova, V., Yordanova, V., Kostadinova, A., Angelova, M.I., Tessier, C., Nuss, P., Staneva, G., Structural Changes Induced by Resveratrol in Monounsaturated and Polyunsaturated Phosphatidylcholine-Enriched Model Membranes, *Membranes*, 2023, 13(12), 909; PMID: 38132913; PMCID: PMC10744944; (IF_{2023}= 4.2, Q2) DOI: 10.3390/membranes13120909;
https://pubmed.ncbi.nlm.nih.gov/38132913/

Abstract: Resveratrol (Resv) is considered to exert a beneficial impact due to its radical scavenger, anti-microbial and anti-inflammatory properties through several mechanisms that could include its interaction with the cell plasma membrane. To address this issue, we investigated the influence of Resv on membrane lipid order and organization in large unilamellar vesicles composed of different lipids and ratios. The studied lipid membrane models were composed of phosphatidylcholine (PC) species (either palmitoyl-docosahexaenoyl phosphatidylcholine (PDPC) or palmitoyl-oleoyl phosphatidylcholine (POPC)), sphingomyelin (SM) and cholesterol (Chol). This study found that the addition of Resv resulted in complex membrane reorganization depending on the degree of fatty acid unsaturation at the *sn-2* position, and the Lipid/Resv and SM/Chol ratios. Resv rigidified POPC-containing membranes and increased liquid-ordered (L_o) domain formation in 40/40/20 POPC/SM/Chol mixtures as this increase was lower at a 33/33/34 ratio. In contrast, Resv interacted with PDPC/SM/Chol mixtures in a bimodal manner by fluidizing/rigidifying the membranes in a dose-dependent way. L_o domain formation upon Resv addition occurred via the following bimodal mode of action: L_o domain size increased at low Resv concentrations; then, L_o domain size decreased at higher ones. To account for the variable effect of Resv, we suggest that it may act as a "spacer" at low doses, with a transition to a more "filler" position in the lipid bulk. We hypothesize that one of the roles of Resv is to tune the lipid order and organization of cell plasma membranes, which is closely linked to important cell functions such as membrane sorting and trafficking.

6.[B4] Vitkova, V., **Hazarosova, R.**, Valkova, I., Momchilova, A., Staneva, G., Glycerophospholipid Polyunsaturation Modulates Resveratrol Action on Biomimetic Membranes, *Colloids and Surfaces B: Biointerfaces*, 2024, 238:113922; (IF_{2024}=5.71, Q1) PMID: 38678790; DOI: 10.1016/j.colsurfb.2024.113922;
https://pubmed.ncbi.nlm.nih.gov/38678790/

Abstract: The phytoalexin resveratrol has received increasing attention for its potential to prevent oxidative damages in human organism. To shed further light on molecular mechanisms of its interaction with lipid membranes we study resveratrol influence on the organisation and mechanical properties of biomimetic lipid systems composed of synthetic phosphatidylcholines with mixed aliphatic chains and different degree of unsaturation at *sn-2* position (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, POPC, and 1-palmitoyl-2-docosahexaenoyl-sn-glycero3-phosphocholine, PDPC). High-sensitivity isothermal titration

calorimetric measurements reveal stronger spontaneous resveratrol association to polyunsaturated phosphatidylcholine bilayers compared to the monounsaturated ones resulting from hydrophobic interactions, conformational changes of the interacting species and desolvation of molecular surfaces. The latter is supported by the results from Laurdan spectroscopy of large unilamellar vesicles providing data on hydration at the glycerol backbones of glycerophospholipides. Higher degree of lipid order is reported for POPC membranes compared to PDPC. While resveratrol mostly enhances the hydration of PDPC membranes, increasing POPC dehydration is reported upon treatment with the polyphenol. Dehydration of the polyunsaturated lipid bilayers is measured only at the highest phytoalexin content studied (resveratrol/lipid 0.5 mol/mol) and is less pronounced than the effect reported for POPC membranes. The polyphenol effect on membrane mechanics is probed by thermal shape fluctuation analysis of quasispherical giant unilamellar vesicles. Markedly different trend of the bending elasticity with increasing resveratrol concentration is reported for the two types of phospholipid bilayers studied. POPC membranes become more rigid in the presence of resveratrol, whereas PDPC-containing bilayers exhibit softening at lower concentrations of the polyphenol followed by a slight growth without bilayer stiffening even at the highest resveratrol content explored. The new data on the structural organization and membrane properties of resveratrol-treated phosphatidylcholine membranes may underpin the development of future liposomal applications of the polyphenol in medicinal chemistry.

Група от показатели Г

Показател Г7: Научни публикации в издания, които са реферирани и индексирани в световноизвестни бази данни с научна информация (Web of Science и Scopus)

1.[Г7] Pankov, R., Markovska, T., **Hazarosova, R.**, Antonov, P., Ivanova L., Momchilova A., Cholesterol Distribution in Plasma Membranes of b1 Integrin-expressing and b1 Integrin-deficient Fibroblasts, *Archives of Biochemistry and Biophysics*, 2005, 442(2), 160-168; (IF_{2005}= 3.165, Q1)
PMID: 16165083; DOI: 10.1016/j.abb.2005.08.003;
https://pubmed.ncbi.nlm.nih.gov/16165083/

Abstract: The effect of integrin receptors on the level and transmembrane localization of cholesterol molecules was investigated in β1 integrin-expressing (β1) and β1 integrin-deficient (β1 null) cells. We found that the content of specific raft components-cholesterol, sphingomyelin, and caveolin-was increased in integrin-expressing cells. Integrin presence affected as well the transmembrane distribution of cholesterol-a higher percent was found in the

plasma membrane outer monolayer of β1 compared to β1 null cells. Sphingomyelin depletion reduced the presence of cholesterol in the outer membrane monolayer of both cell lines, but the differences in cholesterol asymmetry, observed between β1 and β1 null cells before sphingomyelinase treatment were preserved. These findings implied that integrin receptors affected the non-random transmembrane distribution of cholesterol. Finally, a higher percent of detergent-resistant membranes was obtained from β1 integrin-expressing cells, suggesting that the presence of these receptors in the membranes influenced the formation and/or stabilization of lipid raft domains.

2.[Г7] Topouzova-Hristova, T., **Hazarosova, R.**, Bandreva, B., Stephanova, E., Halothane does not Directly Interact with Genome DNA of A549 Cells, *Folia Biol (Praha)*, 2007, 53(5), 176-82; (IF_{2007}=0.596, Q3)
PMID: 17976308;
https://pubmed.ncbi.nlm.nih.gov/17976308/

Abstract: Although the inhalation anaesthetics are commonly used in clinical practice, their toxic effects on the lung cells have not yet been well studied. Previous studies indicated strong genotoxic effect of some inhalation anaesthetics, applied at clinically relevant concentrations. The aim of the present study was to assess the extent of DNA damage, nuclear abnormalities and possibility of human A549 cells to recover after treatment with halothane at lower concentrations. The data obtained demonstrate that even lower halothane concentrations could induce DNA damage although the anaesthetic does not interact directly with DNA. We have found that irreversible impairment of the cell genome is initiated at a concentration as low as 1.5 mM. Part of the cell population displays some characteristics of stress-induced apoptosis, defining this concentration as threshold for cell survival. We suggest that the intracellular signalling pathway triggers the toxic effects of halothane.

3.[Г7] Stephanova, E., Topouzova-Hristova, T., **Hazarosova, R.**, Moskova, V., Halothane-induced Alterations in Cellular Structure and Proliferation of A549 Cells, *Tissue and Cell*, 2008, 40(6), 397-404; (IF_{2008}=0.738, Q2)
PMID: 18508102; DOI: 10.1016/j.tice.2008.04.001;
https://pubmed.ncbi.nlm.nih.gov/18508102/

Abstract: Genotoxicity, cytotoxicity or teratogenicity are among the well-known detrimental effects of the volatile anaesthetics. The aim of the present work was to study the structural changes, proliferative activity and the possibility of alveolar A549 cells to recover after *in vitro* exposure to halothane at 1.5 and 2.1 mM concentrations. Our data indicated significant reduction of viability, suppression of mitotic activity more than 60%, and that these alterations were accompanied by disturbances of nuclear and nucleolar structures. The most prominent negative effect was the destruction of the lamellar bodies, the main storage organelles of pulmonary surfactant, substantial for the lung physiology. In conclusion, halothane applied at clinically relevant concentrations exerts genotoxic and cytotoxic effect on the alveolar cells *in vitro*, most

likely as a consequence of stress-induced apoptosis, thus modulating the respiratory function.

4.[Г7] Lupanova, T., **Hazarosova, R.**, Georgieva, R., Momchilova, A., Staneva, G., Effect of palmitoyl-oxovaleroyl phosphatidylcholine on raft-like domain formation in giant unilamellar vesicles, *Comptes rendus de l'Acade'mie bulgare des Sciences*, 2012, 65(12), 1691-1694; (IF_{2012}= 0.284, Q2)
ISSN: 13101331;
https://www.researchgate.net/publication/288237321_Effect_of_palmitoyl-oxovaleroyl_phosphatidylcholine_on_raft-like_domain_formation_in_giant_unilamellar_vesicles

Abstract: The influence of the oxidized phospholipid palmitoyl-oxovaleroyl-phosphocholine (POVPC) on membrane lateral organization was investigated using fluorescence microscopy. Giant unilamellar vesicles (GUVs) composed of palmitoyldocosahexaenoyl-phosphocholine (PDPC)/egg sphingomyelin (eggSM)/cholesterol (CHOL) mixtures were used as a model system mimicking cellular rafts in biological membranes. The effect of POVPC on the raft-like domain formation was studied. PDPC/SM/CHOL mixtures displayed liquid-disordered (L_d)/liquid-ordered (L_o) phase separation in a large temperature range, from 66°C to 24°C. The presence of PDPC increased the temperature of raft-like domain formation and the fraction of L_o phase. POVPC also induced budding of L_d domains.

5.[Г7] Yordanova, V., Staneva, G., Vitkova, V., Angelova, M., Kostadinova, A., Benkova, D., Veleva, R., Nesheva, A., **Hazarosova, R.**, Biomimetic Vesicles as a Tool to Reveal the Physicochemical Membrane Changes Induced by Oxidised Lipids, *Oxidation Communications*, 2020, 43(4), 678 – 687; (SJR_{2020}=0.224, Q3); ISSN: 02094541;
https://openurl.ebsco.com/EPDB%3Agcd%3A13%3A26365613/detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A147990808&crl=c

Abstract: The oxidation of lipids has been shown to impact all cellular processes. However, the molecular level influence of oxidised lipids on the structure and function of cell membranes remains largely elusive. The formation of membrane lipid domains (rafts) in cells has attracted much attention because of its implications in membrane-related processes. Raft-like domains in model membranes represent a liquid-ordered (L_o) phase, enriched in sphingomyelin (SM) and cholesterol (Chol) surrounded by continuous liquid-disordered (L_d) phase, mainly composed of unsaturated phosphatidylcholine (PC) molecules. In the present study, we examined the effect of oxidised lipid 1-palmitoyl-2-(5′-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) on the lipid order in the PC/SM/Chol ternary lipid mixture systems (model of L_o /L_d coexistence) by using the biophysical experimental method Laurdan fluorescence spectroscopy of large unilamellar vesicles (LUVs). Two hetero-acid glycerophosphocholines, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (PDPC), were used to examine the physicochemical changes in membrane lateral organisation induced by POVPC as a function of the degree of unsaturation of fatty acids at *sn-2* position of PC molecule. We ascertained that POVPC induced larger structural membrane changes in monounsaturated (POPC) lipid matrix compared to polyunsaturated (PDPC) one. The bending rigidity of POVPC enriched membranes was probed via fluctuation spectroscopy of

giant unilamellar vesicles (GUVs), testifying to the strong softening effect of the oxidised lipid on the monounsaturated (POPC) membranes. The obtained results could reveal the molecular mechanisms of POVPC action on the membrane 2D and 3D remodelling.

6.[Г7] Kostadinova, A., Staneva, G., Benkova, D., Yordanova, V., **Hazarosova, R.**, Veleva, R., Nesheva, A., Momchilova, A., Yankova, R., Elzorkany, H., Elshoky, H., Interactions of Chitosan-based Nanoparticles with Bio-inspired Membranes, *Oxidation Communications*, 2021, 44(1), 63-71;
(SJR_{2021}=0.224, Q3)
DOI: 10.1016/j.ijbiomac.2024.133983;
https://openurl.ebsco.com/EPDB%3Agcd%3A4%3A26365636/detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A149752237&crl=f

Abstract: Chitosan is a natural copolymer derived from the deacetylation of chitin. Because of its specific physicochemical properties, chitosan is a perfect material to be used in bioengineering and biomedicine. In this study, we demonstrated how chitosan is able to affect the lipid order and organisation in biomimetic membranes. We formed large unilamellar vesicles (LUVs) composed of different lipids and their mixtures mimicking the lipid architecture of mammalian plasma membranes. Lipid order was probed by Laurdan spectroscopy measurements at physiological temperature. We studied LUVs composed of egg phosphatidylcholine (ePC) exhibiting lipid bilayer in liquid-disordered phase (L_d), egg sphingomyelin (eSM)/cholesterol (Chol) in liquid-ordered phase (L_o), and ePC/eSM/Chol mixtures representing L_d/L_o phase coexistence. The ternary mixture compositions mimic the plasma membrane organisation and formation of raft-like domains with different sizes. Membrane rafts are known to be involved in crucial cellular physiological events in health and disease. LUVs were treated by increasing chitosan concentration and lipid order was assessed by Laurdan fluorescence spectroscopy. The interaction of chitosan with lipid membranes induces an increase in the lipid order independently of the lipid phase state. The highest increase in the lipid order was observed for the L_d phases, whereas, the lowest one was detected for the L_o phase. We suggested molecular mechanisms of interaction of chitosan with the main lipid classes and their phase state.

7.[Г7] Yordanova, V., Staneva, G., Angelova, M., Vitkova, V., Kostadinova, A., Benkova, D., Veleva, R., **Hazarosova, R.**, Modelling of Molecular Mechanisms of Membrane Domain Formation during the Oxidative Stress: Effect of Palmitoyl-oxovaleroyl-phosphatidylcholine, *Comptes rendus de l'Académie bulgare des Sciences*, 2021, 74(1), 78-87;
(IF_{2021}=0.343, Q2)
ISSN: 13101331; DOI: 10.7546/CRABS.2021.01.10;
https://www.researchgate.net/publication/354254510_Modelling_of_molecular_mechanisms_of_membrane_domain_formation_during_the_oxidative_stress_Effect_of_palmitoyloxovaleroyl-phosphatidylcholine

Abstract: The study is focused on the effect of the oxidized lipid 1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) on the physicochemical properties of model membranes using two biophysical experimental methods: fluorescence video-microscopy and fluorescence spectroscopy. Two types of ternary mixtures made of sphingomyelin (SM),

cholesterol (Chol) and respectively, 1-palmitoyl-2-oleoyl sn-glycero-3-phosphocholine (POPC) or 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (PDPC) were used as a model system mimicking the lipid composition of specialized cellular membrane domains, called rafts. The monounsaturated POPC and polyunsaturated PDPC permit revealing the effects of the degree of unsaturation at *sn-2* position in the phosphatidylcholine (PC) species. Fluorescence microscopy showed a difference in the effect of POVPC on raft-like domain formation in POPC/SM/Chol and PDPC/SM/Chol mixtures. In the PDPC mixtures, the oxidized lipid increased the temperature of micron-scale L_o (liquid-ordered)/L_d (liquid-disordered) phase separation whereas in the POPC-containing ones, POVPC abolishes the phase separation. The results from fluorescence spectroscopy, giving information about the nano-scale L_o/L_d phase separation, demonstrate that POVPC promotes the raft-like domain formation in a stronger dose-dependent way in monounsaturated lipid matrix compared to the polyunsaturated one. Fluctuation spectroscopy revealed the strong softening of POPC bilayers upon addition of only 5 mol% of POVPC. We ascertained the fact that the oxidized lipid, POVPC, induced larger structural changes in the membrane organization of monounsaturated lipid matrix. Thus, the results describe a structurally protective role of docosahexaenoic acid against the presence of the oxidized lipid.

8.[Г7] Yordanova, V., **Hazarosova, R.**, Vitkova, V., Kostadinova, A., Angelova, M., Momchilova, A., Krastev, P., Staneva, G., Oxidized Lipids Control Lipid Order and Phospholipase A2 Activity in Model Membranes, *Comptes rendus de l'Académie bulgare des Sciences*, 2022, 75(4), 581-589; (IF_{2022}=0.326, Q3)
ISSN: 13101331; DOI: 10.7546/CRABS.2022.04.13;
https://www.proceedings.bas.bg/index.php/cr/article/view/68

Abstract: Oxidative stress is an important etiologic factor in the pathogenesis of various diseases. The formation of oxidized phospholipid species in vivo induces membrane remodelling with direct pathological implications with a prominent inflammatory component. Secretory phospholipases A2 (sPLA2) are involved in the regulation of inflammation and immune response. Their activity is highly dependent on the lipid membrane composition, structure and organization. In this work, we studied the impact of oxidized phosphatidylcholines (OxPCs) on the membrane lipid order and the sPLA2 activity. The effects of two of the most physiologically active OxPCs, 1-palmitoyl-2-(5′-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-glutaroyl-sn-glycero-3- phosphocholine (PGPC) were compared using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) vesicles. Both OxPCs reduce the membrane lipid order and sPLA2 activity at physiological temperature. Moreover, these changes depend on the chemical nature of the oxidized chains.

9.[Г7] Vitkova, V., Staneva, G., **Hazarosova, R.**, Georgieva, S. I., Valkova, I., Antonova, K.,

Todorov, P., Valorphins Alter Physicochemical Characteristics of Phosphatidylcholine Membranes: Datasets on Lipid Packing, Bending Rigidity, Specific Electrical Capacitance, Dipole Potential, Vesicle Size, *Data in Brief*, 2022, 45, 108716; (IF_{2022}=1.38, Q4)
PMID: 36426033; PMCID: PMC9679672; DOI: 10.1016/j.dib.2022.108716;
https://pubmed.ncbi.nlm.nih.gov/36426033/

Abstract: Endogenous hemorphins are being intensively investigated as therapeutic agents in neuropharmacology, and also as biomarkers in mood regulation, inflammation and oncology. The datasets collected herein report physicochemical parameters of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine membranes in the presence of VV hemorphin-5 (Val-Val-Tyr-Pro-Trp-Thr-Gln) and analogues, modified at position 1 and 7 by the natural amino acid isoleucine or the non-proteinogenic 2-aminoisobutyric, 2,3-diaminopropanoic or 2,4-diaminobutanoic amino acids. These peptides have been previously screened for nociceptive activity and were chosen accordingly. The present article contains fluorescence spectroscopy data of Laurdan- and di-8-ANEPPS labelled large unilamellar vesicles (LUV) providing the degree of hydration and dipole potential of lipid bilayers in the presence of VV-hemorphin-5 analogues. Lipid packing is accessible from Laurdan intensity profiles and generalized polarization datasets reported herein. The data presented on fluorescence intensity ratios of di-8-ANEPPS dye provide dipole potential values of phosphatidylcholine-valorphin membranes. Vesicle size and electrophoretic mobility datasets included refer to the effect of valorphins on the size distribution and ζ -potential of POPC LUVs. Investigation of physicochemical properties of peptides such as diffusion coefficients and heterogeneous rate constant relates to elucidation of transport mechanisms in living cells. Voltammetric data of valorphins are presented together with square-wave voltammograms of investigated peptides for calculation of their heterogeneous electron transfer rate constants. Datasets from the thermal shape fluctuation analysis of quasispherical 'giant' unilamellar vesicles (GUV) are provided to quantify the in fluence of hemorphin incorporation on the membrane bending elasticity. Isothermal titration calorimetric data on the thermodynamics of peptide-lipid interactions and the binding affinity of valorphin analogues to phosphatidylcholine membranes are reported. Data of frequency-dependent deformation of GUVs in alternating electric field are included together with the values of the specific electrical capacitance of POPC-valorphin membranes. The datasets reported in this article can underlie the formulation and implementation of peptide-based strategies in pharmacology and biomedicine.

10.[Г7] Vitkova, V., Staneva, G., **Hazarosova, R.**, Georgieva, S. I., Valkova, I., Antonova, K., Todorov, P., Interaction of New VV-hemorphin-5 analogues with Cell Membrane Models, *Colloids and Surfaces B-Biointerfaces*, 2022, 220, 112896; (IF_{2022}=5.88, Q1)
PMID: 36270140 DOI: 10.1016/j.colsurfb.2022.112896;
https://pubmed.ncbi.nlm.nih.gov/36270140/

Abstract: New analogues of the endogenous heptapeptide VV-hemorphin-5 (valorphin) synthesised by amino acid replacement allow for tailoring the peptide activity in vivo. Investigation of hemorphin-induced alterations of lipid bilayers' physicochemical parameters unravels membrane-mediated mechanisms of interaction with cells and subcellular structures.

We studied the effect of modified valorphins with nociceptive activity on the structure, mechanical and electrical properties of lipid membrane models. Lower bending rigidity and higher specific capacitance of phosphatidylcholine bilayers were found in the presence of VV-hemorphin-5 analogues. Peptide partition constants for the transfer from the aqueous solution into the membrane were determined by isothermal titration calorimetry. It was found that the inclusion of non-proteinogenic acids with different number of methylene groups lead to alterations of hemorphin-membrane binding. The highest membrane affinity was obtained for a hemorphin derivative with dose-dependent variable effects on visceral nociception in mice. The valorphin analogue with the most pronounced anti-nociceptive effect in vivo induced the highest dipole and zeta potential change without significantly affecting the lipid packing at glycerol level in phosphatidylcholine bilayers.

11.[Г7] Ivanova, I., Toshkovska, R., Yocheva, L., Benkova, D., Yordanova, V., Nesheva, A., **Hazarosova, R.**, Staneva, G., Kostadinova, A., Stress Response of Gram-Positive and Gram-Negative Bacteria Induced by Metal and Non-metal Nanoparticles. In Search of Smart Antimicrobial Agents, *Lecture Notes in Networks and Systems*, 2nd International Symposium on Bioinformatics and Biomedicine, BioInfoMed 2022, Burgas, 658 LNNS, 147-155, 2023; (SJR_{2023}=0.150, Q4)
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Abstract: The increasing resistance of pathogens to a number of antibiotics has been the subject of many research reports from the European Antibiotic Resistance Surveillance Network (Ears-Net) and The World Health Organization (The WHO). The aim of this work was to study the effects of nanomaterial dispersions as Selenium (Se), Gold (Au), Iron oxide (Fe_2O_3), Silicon dioxide (SiO_2) and Graphene oxide (GO) on bacteria like Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus and two strains of Escherichia coli. Two classical methods were used to investigate the antibacterial effect of the nanoparticles (NPs): Spot and Well diffusion tests in agar medium. The tested nanoparticles were active against Gram-positive bacteria in concentrations between 3.0 and 1.5 mg/mL but they were not active against Gram-negative bacteria such as E. coli. Among tested nanomaterials, Se NPs express the strongest antimicrobial effect. Gold nanoparticles with Polyvinylpyrolidone (Au-PVP NPs) were more active against bacteria than pure Au NPs. Lower concentrations (1.0 mg/mL and 0.5 mg/mL) of Se, GO and the two types of Gold nanoparticles did not show activity against all test microorganisms. Fe_2O_3 NPs as well as SiO_2 NPs had no effect on any test bacteria in the mentioned concentrations.
In conclusion, the most cytotoxic for tested bacteria were Se NPs, followed by Au-PVP and Au NPs. GO NPs also showed a certain cytotoxic effect, especially on B. cereus.

12.[Г7] Yordanova, V., Staneva, G., Krastev, P., Markovska, T., Marinovska, A., Kostadinova, A., **Hazarosova, R.**, Momchilova, A., Lipid Order of Membranes Isolated from Erythrocytes of

Patients with Coronary Artery Disease: Correlation with Biochemical Parameters, *Lecture Notes in Networks and Systems*, 2nd International Symposium on Bioinformatics and Biomedicine, BioInfoMed 2022, Burgas, 658 LNNS, 134-146, 2023, (SJR_{2023}=0.150, Q4)
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Abstract: Coronary artery disease (CAD) is a medical condition that is characterized by an inability of the arteries to supply the myocardium of the heart with enough blood, therefore affecting its function and leading to serious pathological changes. CAD development is caused by plaque buildup on the inner walls of the coronary arteries. The plasma and red blood cell lipids are involved in plaque formation. In this study, the fatty acid (FA) composition was determined in blood plasma and membranes isolated from erythrocytes of treated patients against CAD and healthy controls. Higher saturated/unsaturated FA ratio was found in the erythrocyte ghost membranes of patients compared to controls. Moreover, the triglyceride (TG) levels of CAD patients were also higher compared to the control group. No difference in total cholesterol and LDL-cholesterol levels was observed between patients and control group. Unexpectedly, despite the observed higher level of saturated FAs in the erythrocyte membranes, lower membrane lipid order was found for CAD group compared to the control one. Our results suggest that the serum TGs govern more significantly the lipid order of CAD ghost membranes than the saturated/unsaturated FA ratio. This finding is in accordance with the literature data stating an inverse relationship between TG levels and the lipid anisotropy in model and erythrocyte membranes. The present study implies that CAD treatment is able to decrease erythrocyte membrane lipid order, whereas higher levels of TGs in blood serum and saturated FAs in erythrocyte membranes still represent a critical potential risk for CAD patients compared to healthy individuals.

13.[Г7] Yordanova, V., **Hazarosova, R.**, Vitkova, V., Momchilova, A., Robev, B., Nikolova, B., Krastev, P., Nuss, P., Angelova, M.I., Staneva, G., Impact of Truncated Oxidized Phosphatidylcholines on Phospholipase A2 Activity in Mono- and Polyunsaturated Biomimetic Vesicles, *International Journal of Molecular Sciences*, 2023, 24(13), 11166; (IF_{2023}=5.6, Q1)
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Abstract: The interplay between inflammatory and redox processes is a ubiquitous and critical phenomenon in cell biology that involves numerous biological factors. Among them, secretory phospholipases A2 (sPLA2) that catalyze the hydrolysis of the *sn-2* ester bond of phospholipids are key players. They can interact or be modulated by the presence of truncated oxidized phosphatidylcholines (OxPCs) produced under oxidative stress from phosphatidylcholine (PC) species. The present study examined this important, but rarely considered, sPLA2 modulation induced by the changes in biophysical properties of PC vesicles comprising various OxPC ratios in mono- or poly-unsaturated PCs. Being the most physiologically active OxPCs, 1-palmitoyl-2-(5′-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-glutaryl-sn-glycero-

3-phosphocholine (PGPC) have been selected for our study. Using fluorescence spectroscopy methods, we compared the effect of OxPCs on the lipid order as well as sPLA2 activity in large unilamellar vesicles (LUVs) made of the heteroacid PC, either monounsaturated [1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)], or polyunsaturated [1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (PDPC)] at a physiological temperature. The effect of OxPCs on vesicle size was also assessed in both the mono- and polyunsaturated PC matrices. Results: OxPCs decrease the membrane lipid order of POPC and PDPC mixtures with PGPC inducing a much larger decrease in comparison with POVPC, indicative that the difference takes place at the glycerol level. Compared with POPC, PDPC was able to inhibit sPLA2 activity showing a protective effect of PDPC against enzyme hydrolysis. Furthermore, sPLA2 activity on its PC substrates was modulated by the OxPC membrane content. POVPC down-regulated sPLA2 activity, suggesting anti-inflammatory properties of this truncated oxidized lipid. Interestingly, PGPC had a dual and opposite effect, either inhibitory or enhancing on sPLA2 activity, depending on the protocol of lipid mixing. This difference may result from the chemical properties of the shortened *sn-2*-acyl chain residues (aldehyde group for POVPC, and carboxyl for PGPC), being, respectively, zwitterionic or anionic under hydration at physiological conditions.