Globustar Formaat
Tallinn, Estonia, 2021

CTP PCP RAS, MOSCOW (RUSSIA)

AUG 25-27, 2021

SYSTEMS BIOLOGY AND SYSTEMS PHYSIOLOGY: REGULATION OF BIOLOGICAL NETWORKS

THE MEETING IS DEDICATED TO 75TH ANIVERSARY OF FAZLY ATAULLAKHANOV

HYBRID CONFERENCE 2021 ABSTRACTS

EDITED BY: MIKHAIL A. PANTELEEV AND ALEXANDRA A. FILKOVA

Title: Systems Biology and Systems Physiology: Regulation of Biological Networks (devoted to the 75th Anniversary of Fazly Ataullakhanov), 2nd Hybrid Conference Proceedings

Hosted by: Centre For Theoretical Problems of Physicochemical Pharmacology RAS, Moscow, Russian Federation; 25 August 2021 – 27 August 2021

Editors: Prof. Dr. Mikhail A. Panteleev, Dr. Fazly I. Ataullakhanov and Alexandra A. Filkova

Corrections: Alexey A. Martyanov

Artwork by: Ekaterina M. Koltsova

ISBN:

Published by: Globustar Formaat OU, Tallinn, Estonia, 2021; https://sbpreports.com/

Printed: Online-only publication

Editor-in-chief

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Contents

Topic: Cell division and molecular motors

The Tip of a Growing Microtubule and its Implications for Polymer Dynamics

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Background: The dynamics of microtubules (MTs) is important for the organization, motility, and morphogenesis of cells. Most tubulin subunits join and leave a MT at its plus tip, so the structure of this site is informative about intermediates in tubulin polymerization. Moreover, a dynamic MT is a machine that can push or pull on cellular objects, changing their position. The force a growing MT can exert is modest (~2pN) whereas shortening MTs can pull hard (~30 pN). Cells may use these capabilities to deform membranes, move organelles, and to organize then segregate the chromosomes in preparation for cell division.

Aims: We have studied the structure of the tips of growing and shortening MTs, both in cells and in test tubes, to learn about the changes in structure that tubulin experiences as it polymerizes and depolymerizes.

Methods: Cells were embedded in plastic, sectioned and imaged by electron tomography, a method that provides 3-D images of structures with resolutions of ~4 nm. We have also used this and related imaging methods to study MTs growing in vitro that were rapidly frozen, MTs that were fixed and then frozen, and MTs that were fixed or not, then negatively stained.

Results: The growing MTs in our images almost all displayed flared ends in which protofilaments (PFs, i.e. linear strands of tubulin) bent outward from the MT axis. Most PFs lay in planes that contained the MT axis. PF curvature was greatest at its tip and decreased linearly with approach to the MT wall. Tip PF curvature resembled the curvature of isolated tubulin as measured by several crystallographers. Analysis of the structural data by Nikita Gudimchuk and colleagues, using Brownian dynamics, has demonstrated that thermal fluctuations can provide sufficient movement to let the PFs straighten, find neighbors, and form lateral bonds to zip the PFs into the elongating MT wall. Model fitting to published data on rates of MT growth and the forces generated by dynamic MTs provide parameter values that are reasonable for both the strength and activation energies of all inter-tubulin bonds.

Conclusions: We conclude that MTs polymerize by the addition of curved tubulin dimers onto the tips of bent PFs, which straighten by thermal motions to allow MT elongation. This simple model solves several enigmas about tubulin dynamics and allows straightforward interpretation of the synergistic activity of factors that enhance the rate of MT growth. There are, however, unsolved problems, such as how tubulin's structure changes during polymerization to yield PFs with linear changes in their curvature.

Permitted and restricted steps of human kinetochore assembly in mitotic cell extracts

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Background: Assembly of kinetochore and its proper interaction with spindle microtubules are critical steps for accurate chromosome segregation. In human cells, assembly of outer kinetochore layers occurs during mitosis based on two major constitutive proteins CENP-C and CENP-T, which recruit main kinetochore microtubule binder Ndc80 complex and other kinetochore components. Hierarchical recruitment of kinetochore proteins from cytoplasmic pool is a highly regulated process, but the underlying steps are not well understood. Also, it is not clear how different kinetochore proteins contribute to interaction with spindle microtubules.

Aims: The ultimate goal of our research is to gain understanding of mechanisms driving kinetochore assembly and interaction with microtubules in mitosis.

Methods: We developed assembly assays to monitor the recruitment of GFP-tagged recombinant proteins and native proteins from human cell extracts to inner kinetochore components immobilized on microbeads.

Results: In contrast to prior work in yeast and Xenopus egg extracts, we find that human mitotic cell extracts fail to support de novo assembly of microtubule-binding sub-complexes. A subset of interactions, such as those between CENP-A–containing nucleosomes and CENP-C, are permissive under these conditions. However, the subsequent phospho-dependent binding of the Mis12 complex is less efficient, whereas recruitment of the Ndc80 complex is blocked, leading to weak microtubule-binding activity of assembled particles. Using molecular variants of the Ndc80 complex, we show that auto-inhibition of native Ndc80 complex restricts its ability to bind to the CENP-T/W complex, whereas inhibition of the Ndc80 microtubule-binding is driven by a different mechanism.

Conclusions: Together, our work reveals regulatory mechanisms that guard against the spurious formation of cytosolic microtubule-binding kinetochore particles.

Molecular dynamic model of NDC80 complex interaction with a microtubule

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Background: In the process of cell division, kinetochores interact with microtubules, resulting in the separation of sister chromosomes. One of the key elements kinetochore-microtubule attachment is the NDC80 protein complex.

Aims: Establish the dynamic and structural properties of the NDC80 complex and its interaction with the microtubule using molecular modeling methods.

Methods: We used coarse-grained explicit solvent molecular dynamics calculations to simulate the behavior of the NDC80 complex dissociation under applied force. In our MARTINI model, one coarse-grain particle replaced a group of, on average, 4 heavy atoms with hydrogen atoms associated with them, which allowed us to get rid of the fastest molecular degrees of freedom and, accordingly, increase the integration step of the molecular dynamics equations. This approach makes it possible to accelerate molecular dynamics calculations up to 2 orders of magnitude. As a result, MARTINI 3 coarse-grain force-field which is optimal for protein simulation allowed us to study the process of NDC80 complex dissociation induced by force at almost atomic resolution at the microsecond timescale. For molecular dynamics calculations we used the Gromacs software package.

Results: Computational experiments of the force-induced dissociation of the NDC80 complex with the application of forces of 9 pN, 16 pN, and 25 pN along the microtubule axis towards its plus and minus ends were carried out. These experiments have shown that the binding force is higher when a force is applied towards the plus-end, compared to movement towards the minus-end. This asymmetry of diffusion of the NDC80 complex along a microtubule under the action of a mechanical force may arise due to the initial orientation of the supercoiled section at an angle toward the plus-end of the microtubule. Besides, the contact area between the NDC80 and the microtubule significantly expands upon relaxation of the complex due to the formation of additional contacts of tubulin with Nuf2 protein, reinforcing the asymmetry of the NDC80 complex motion along the microtubule under the influence of mechanical force.

Conclusions: Our study offers an explanation of the asymmetry of diffusion of the NDC80 complex along a microtubule under force, recently experimentally discovered by the group of prof. F.I. Ataullakhanov and co-authors. This work was supported by the Interdisciplinary Scientific and Educational School of Moscow University “Photonic and Quantum Technologies. Digital Medicine”.

The ratio of Tpm α- and β-chains in the thin filament affects the actin-myosin interaction

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Background: Tropomyosin (Tpm) is a regulatory protein that controls the actin-myosin interaction in striated muscle. The TPM1 and TPM2 genes encode respectively the α- and β-chains of Tpm, which are identical by 87%. The α- and β-chains of Tpm form αα- and ββ-homodimers and also αβ-heterodimer. The αα-homodimer and αβ-heterodimer are essentially more stable than ββ-homodimer, and so most mammalian striated muscles contain predominantly αα- and αβ-dimer (Boussouf et al., 2007). Expression of α- and β-chains in the muscle depends upon the species and age of the animal and may change in pathologies. Thus, the cardiac muscle of small mammals contains solely αα-Tpm homodimer, while the heart of large mammals also contains αβ-heterodimer.

Aims: This work aimed to estimate the effect of the ratio of Tpm α- and β-chains in the thin filament composition on functional properties of the actin-myosin interaction.

Methods: Using a two-beam optical trap, we studied how different Tpm isoforms affect the average step size, the force developed by a single myosin molecule interacting with thin filaments (filamentous actin, Tpm, and troponin complex), durations of the interaction with and without workload, and also bending stiffness of the thin filament. Using an in vitro motility assay, we evaluated the effects of Tpm isoforms on the maximal sliding velocity of reconstructed thin filaments, containing corresponding Tpm isoforms.

Results: Compared to F-actin, all Tpm isoforms greatly increased the bending stiffness of thin filaments and their sliding velocity along the myosin-coated surface measured in the in vitro motility assay. The presence of β-chain reduced both these parameters as compared to αα-Tpm. We found no reliable difference in the myosin step size along thin filaments with the studied Tpm isoforms.

Conclusions: We conclude that in comparison with αα-Tpm, the presence of β-chain in the Tpm molecule shortens the duration of the actin-myosin interaction both with and without a load, decreases the bending stiffness of reconstructed thin filaments and maximal sliding velocity in the motility assay.

Structure of super-relaxed myosin heads in relaxed muscle

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Background: In a relaxed state in a skeletal or cardiac muscle cell, the globular heads of myosin molecules do not interact with actin but are located near the backbone of a thick filament. Recently the complex nature of this state has been revealed and a new energy-conserving state of myosin called super-relaxed (SRX) has been discovered. Structurally SRX was attributed to an interacting heads motif (IHM) of a pair of myosin heads on the backbone of a thick filament discovered using cryo-electron microscopy. Two heads in the IHM are bound to each other and also have contacts with the neighbourhood.

Aims: Since the cryo-EM technique may cause errors in the positioning of myosin heads on the backbone the correctness of the IHM-based model as a description of the thick filament structure in the relaxed muscle should be verified.

Methods: X-ray diffraction studies of the whole femur muscle of tarantula were combined with the analysis of the diffraction patterns using mathematical modelling.

Results: We compared the intensities of the first six myosin layer lines calculated using IHM-based model with those of the ex vivo and skinned relaxed muscle of tarantula. We also did the

comparison with the densities of the 2.0-nm 3D map, used to fit the 3JBH IHM-based model and the higher resolution (1.3 nm) thick-filament frozen-hydrated 3D maps. The R-factor for the IHM-based model and the ex vivo data was 4.9%, while those for the model and the 2.0- and 1.3-nm 3D-map densities were 4.0% and 8.4%, respectively, indicative of generally good fits.

Conclusions: The correctness of the cryo-EM based IHM model of myosin filaments for relaxed intact and chemically permeabilized muscles has been confirmed.

Role of tropomyosin dynamics in regulation of cardiac muscle in health and disease

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Background: Muscle contraction is powered by the interaction of contractile proteins, myosin and actin that form the thick and thin filaments in the skeletal and cardiac muscles. The contraction-relaxation cycle is controlled by Ca^{2+} ions via regulatory proteins, troponin (Tn) and tropomyosin (Tpm), associated with the thin actin filaments. Tpm is a long coiled-coil protein that polymerises into a long strand on the surface of the thin filament. In the absence of Ca^{2+}, Tn binds actin and keeps the Tpm strand in a blocking position where it prevents myosin binding to actin. Upon Ca^{2+} binding to Tn, the Tpm strand releases from the blocking position, myosin binds actin and generates active force. The Ca^{2+}-regulation depends on many factors including Tpm properties. Theoretical analysis suggests that bending stiffness of Tpm is important for the regulation. Some mutations in Tpm are believed to be associated with myopathies or cardiomyopathies.

Aims: To study the structural and functional role of some conserved Tpm residues and the molecular mechanisms of pathogenicity of some Tpm mutations.

Methods: A combined approach based on an experimental study of structural and functional properties of recombinant Tpm with various amino acid substitutions or posttranslational modification and the molecular dynamics (MD) simulation was employed.

Results: The non-canonical Tpm residues D137 and E218 that destabilize the Tpm coiled-coil are important for its proper regulation of the actin-myosin interaction. The formation of a disulphide bridge between residues Cys190 of two Tpm chains destabilizes the Tpm molecule and might be involved in the development of heart failure upon hypoxia while the phosphorylation (pseudo-phosphorylation) of Tpm residues S283 and S61 mutations can reduce or even eliminate undesirable changes in functional properties of Tpm caused by some cardiomyopathy-associated mutations.

Conclusions: MD simulation is a useful tool for understanding the mechanisms of the regulation of cardiac muscle and its impairment in some genetic cardiomyopathies.

AED Peptide stimulates human stem cell differentiation into dermal fibroblasts

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Background: Peptide regulation of the dermal fibroblasts functions is a new gerontotechnology. Peptide AED reduces the synthesis of proteins p53, p16, caspase-3, MMP9 during ageing of human skin fibroblasts.

Aims: The aim of this work is to evaluate the effect of the AED peptide on gene expression and protein synthesis of early (PDGFRα, Engrailed1) and late (Twist2 and Spry4) differentiation of human stem cells into dermal fibroblasts.

Methods: Human embryonic bone marrow mesenchymal stem cells (line FetMSC) were grown up to the 3rd passage, and the AED peptide (100 ng/ml) or saline solution (control) was added. Quantitative PCR was performed using a qPCRmix-HS SYBR + ROX kit and a DT322 detection amplifier. The concentration of the internal standard (GAPDH mRNA) was taken as a 1. Visual assessment of the synthesis of PDGFRα, Engrailed1, Twist2, Spry4 proteins was performed via immunocytochemistry and immunofluorescence microscopy. The results were statistically processed using the Statistica 10.0 software (Statsoft Inc., Tulsa, USA). Comparison of the mean values of the studied parameter in groups was carried out according to the Student's t-test at a statistical significance level of $p < 0.01$.

Results: The AED peptide significantly increased the expression of the PDGFRα, ENGRAILED1, TWIST2, SPRY4 genes in the FetMSC culture by 2.9, 3.8, 1.4, 1.9 times, respectively, compared to the control. The AED peptide stimulated the synthesis of PDGFRα, Engrailed1, Twist2, Spry4 proteins in the FetMSC culture. After the addition of the AED peptide, 60% of the cells acquired a stellate shape characteristic of fibroblasts, but this was not revealed in the control.

Conclusions: The AED peptide stimulates the expression of genes and synthesis of proteins involved in the differentiation of human skin fibroblasts.

KE and KED peptides regulate PARP and SIRT gene expression during replicative and stationary human mesenchymal stem cells ageing

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Background: Short peptides are involved in the epigenetic regulation of gene expression during cellular ageing. KE peptide has an immunoprotective effect, regulates telomere length of blood lymphocytes and increases animal lifespan. KED peptide possesses vaso- and neuroprotective properties.

Aims: The aim of this work is to study the effect of KE and KED peptides on the expression of PARP-1, PARP-2, PARG, SIRT1 gerontogens in stationary and replicative ageing models of human mesenchymal stem cells of the FetMSC line.

Methods: FetMSC ageing was studied using the Schweigert method with modifications. To simulate replicative senescence, cells were grown up to 7th and 14th passages with the addition of peptides at a concentration of 20 ng/ml. An appropriate volume of saline was added to the control cultures. Quantitative PCR using SYBR Green I dye was performed by means of the QuantiFast SYBR Green PCR Kit (Qiagen, FRG) and a CFX96 Real-Time PCR Detection System (BioRad Laboratories, USA). The results were statistically processed in CFX Manager Software. The GAPDH mRNA was taken as the internal standard; its concentration was taken as a unit. Statistical data analysis was performed according to the two-tailed Student's t test at $p < 0.05$.

Results: KE peptide changed the expression of PARP-1, PARP-2, PARG, SIRT1 genes during replicative and stationary ageing of FetMSC by 1.9-5.5 times. KED peptide changed the expression of PARP-1, PARP-2, PARG, SIRT1 genes during replicative and stationary ageing of FetMSC by 1.9-26.7 times.

Conclusions: KE and KED peptides manifest geroprotective effect in the FetMSC models of replicative and stationary ageing through modulation of the expression of the PARP-1, PARP-2, PARG, SITR1 genes, involved in the regulation of cell repair, differentiation, and apoptosis.

Mechanisms of microtubule dynamic instability examined with Monte-Carlo simulations and in vitro reconstitution experiments

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Background: Microtubules (MTs) are essential tubulin polymers, which dynamic instability behavior manifested in intermittent assembly and disassembly, with relatively rare transitions between these phases, termed catastrophes and rescues. MT dynamic instability helps cells perform numerous functions throughout their life cycle, including chromosome segregation in mitosis. Accumulating biochemical and electron cryotomography evidence has suggested that MTs grow by addition of curved guanosine triphosphate (GTP) tubulins to the tips of curved tubulin protofilaments in vivo and in vitro, challenging previous views about tubulin assembly.

Aims: Here aim to explore the mechanisms of poorly understood MT catastrophes and rescues, building on the recent structural findings about the flared morphology of MT tips

Methods: We formulate a Monte-Carlo model, which explicitly takes into account both the nucleotide and conformational states of tubulin. To test some of the models predictions, we use an in vitro reconstitution approach, comparing dynamic MT behavior when assembled from MT seeds attached to the coverslip or the microfabricated piedestals

Results: Our model can reproduce both MT catastrophes and aging – the gradual decrease of MT stability over time. Analysis of MT dynamics in the presence and absence of soluble tubulin suggests a coupling of GTP hydrolysis to tubulin curvature. When fully constrained, the model predicts no spontaneous rescue events, unless the MT wall contains patches of GTP-tubulin. In a reconstituted system in vitro, we find that the distribution of lengths at which MT rescues occur has a peak located near the nucleating seed. This distribution is closely matched by positions of the GTP-tubulin patches incorporated into the MT body from solution. However, when MTs are assembled from the seeds overhanging from micro-fabricated pedestals, being well separated from the coverslip, their rescue frequency and the GTP islands incidence are substantially reduced.

Conclusions: Our results thus suggest that MT age-dependent catastrophes result both from the tubulin conformation-dependent GTP hydrolysis and from the changes in MT tip configuration over time. Spontaneous rescues of isolated MTs are very rare. The primary mechanism of MT rescue is linked to repair of MT lattice after damage, induced by external factors. This work was partially supported by RF President’s grant to #MK-1869.2020.4 to N.B.G.

Topic: Cellular metabolism and energy

Experimental anticancer compound TIC10 induces transformation of BT474 human breast cancer cells to stable stress-state

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Background: TIC10 (ONC201) – first in class of imipridones experimental drug with potential anticancer efficiency, which demonstrates both cytotoxic and cytostatic effects in variety of cancer cell cultures.

Aims: The aim of our study was to investigate proximate and long-lasting effects of 10μM single dose TIC10 treatment in breast cancer cell line BT474.

Methods: Mammalian cell culture, confocal fluorescent microscopy and image analysis, western blot, RT-PCR, cell cycle analysis by flow cytometry

Results: Using confocal fluorescent microscopy, we quantitatively evaluated the effects of TIC10 on the number mitochondrial nucleoids per cell and mitochondrial morphology. Depending on the duration of treatment, 24 h exposure to TIC10 decreased the number of nucleoids from 249 ± 52 to 155 ± 38 per cell and 72 h exposure further reduced the number of nucleoids to 84 ± 36 per cell, which was associated with fragmentation of mitochondria. The decrease in nucleoid number correlated with the depletion of mtDNA content determined from ND1/ACTB ratio using q-PCR. These effects were accompanied with increased expression of stress-proteins: ATF4, CHOP and GDF-15, assessed by western blot. Nevertheless, we did not observe signs of cytotoxicity, and cells exposed to drug remained viable. Direct counting of cells after treatment with TIC10 using hemocytometer showed that TIC10 significantly inhibits cell proliferation. Flow cytometry analysis of the cell cycle showed accumulation of cells in G0/G1 phase (from 54 ± 13% to 82 ± 2%) with decline of percentage in S phase (from 35 ± 6% to 8 ± 1%) after 72 h treatment with TIC10. Taking together, these observations allowed us to speculate that TIC10 treatment could transform cells rather than killing them.
To confirm drug-induced transformation of cells we removed the drug and continued incubation of cells for 120 h with 24-h media change following drug exposure. Short-term (24 h) treatment with TIC10 followed with 120-h washout resulted in restoration of cell proliferation, partial restoration of nucleoids (214 ± 46 nucleoids/cell) and decline in ATF4, CHOP and GDF-15 expression. On contrary, after 72 hours exposure to TIC10 and subsequent 120-hour washout lead to stable expression of stress-proteins and arrest of proliferation, with further decrease in nuceloids number to 21 ± 11 nucleoids/cell. Despite massive nucleoid depletion and permanent expression of stress-proteins, we did not observe cell death in culture even after washout.

Conclusions: In conclusion, single dose short-term (24 h) exposure of BT474 cells to TIC10 resulted in reversible depletion of mitochondrial nucleoids, inhibition of cell proliferation, and expression of stress proteins as observed after 5 days washout in the absence of the drug. On contrary, single-dose long-term (72 h and longer) exposure of cells to TIC10 induced irreversible, long-lasting effects such as permanent arrest of proliferation with sustained mitochondrial dysfunction and expression of stress proteins, which could be determinants of special stress-phenotype in BT474 cells, induced by TIC10 treatment.

Novel genetically encoded tools for compartment-specific manipulation of NADH/NAD+ and NADPH/NADP+ ratios in living cells

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Background: Emerging evidence attributes cellular reduction-oxidation (redox) imbalance to either the etiology or pathogenesis of primary mitochondrial diseases along with myriad diverse conditions, ranging from cancer and neurodegeneration to aging-associated metabolic changes. However, at present, for most of these conditions it is not known which particular metabolic or signaling pathway, and which cellular compartment, is a major contributor to the redox imbalance or how observed redox imbalance drives a particular pathological phenotype. Most importantly, the key question still stands: Can amelioration of redox imbalance be exploited therapeutically to reverse or arrest pathology? In order to systematically address this question, it is necessary to have tools with which key contributors to the cellular redox environment can be safely and directly modulated with spatial and, most importantly, temporal resolution. Evolutionary adaptations in some bacteria and lower eukaryotes offer attractive possibilities for developing such tools. These organisms use very different strategies for maintaining their optimal redox environment compared to a typical mammalian cell, and therefore these strategies represent a rich source of promising molecular reagents.

Aims: Our overall objective is to develop evolution-inspired, genetically encoded tools for spatiotemporal modulation of cellular redox metabolism in mammalian cells as well as in model organisms.

Methods: We previously reported the use of the naturally occurring Lactobacillus brevis H2O-forming NADH oxidase (LbNOX) as a genetic tool for direct manipulation of the NADH/NAD+ ratio in human cells. We demonstrated that LbNOX alone can fully complement electron transport chain (ETC) function to support mammalian cell proliferation, demonstrating that NAD+ recycling by the respiratory chain is the essential mitochondrial function required for cellular proliferation.

Results: We next turned our attention to using an analogous approach to perturb the NADPH/NADP+ ratio. To our knowledge, a naturally occurring H2O-forming oxidase highly specific for NADPH does not exist. We used rational design and structure-guided rational mutagenesis to engineer a H2O-forming NADPH oxidase, which we call TPNOX. We demonstrated that TPNOX can be safely expressed in different compartments of human cells and can be used to increase cellular NADPH/NADP+ ratio. We subsequently used LbNOX and TPNOX to show that the redox states of mitochondrial NADPH and NADH pools are metabolically connected.

Conclusions: Taken together, the genetically encoded tools we are developing represent a powerful toolkit which can be used to study redox metabolism in normal physiology and disease.

Calcium oscillations and autowaves in cells

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Background: Activation of many receptors causes an intracellular signal in the form of Ca^{2+} oscillations which are auto waves.

Aims: To investigate the properties and functions of calcium autowaves in cells

Methods: fluorescent imaging

Results: Activation of many receptors causes an intracellular signal in the form of Ca^{2+} oscillations. Recording of oscillations in individual cells of the same type showed that the same receptor induces oscillations of different frequencies and amplitudes, and this leads to the expression of different genes. The recording of oscillations in large cells showed that these oscillations are undamped calcium autowaves that propagate along the surface of the endoplasmic reticulum (ER). The mechanism of these auto waves was studied and it was shown that an Ca^{2+} concentration increase at some point activates a Ca^{2+}-dependent Ca^{2+} release from the ER and generates a wave. The same calcium acts as a periodically appearing inhibitor, which closes the channel, interacting with calmodulin. It has also been demonstrated that autowaves can occur when intracellular signaling systems are activated without the

participation of receptors. Thus, the activation of adenylate cyclase caused the chaotic appearance of microwaves in various parts of the cell, the source of which was a local calcium increase, which was followed by the predominance of a single pacemaker source and the appearance of a macro wave. It has been shown that the Ca^{2+} waves are involved in intercellular communication. The intracellular Ca^{2+} wave causes the pulse release of messengers that activate the calcium-mobilizing receptor on neighboring cells. Thus, the intracellular Ca^{2+} wave is transformed into an autowave of extracellular messenger. The voltage-dependence of ionic channels that determine the electrical excitability of nerve cells allows for the existence of a mode of "synchronous" excitation of all neurons in the network. It takes place under the effect of stochastic potential changes in conditions when all neurons are switched to a mode close to excitation. Moreover, if the composition of voltage-generating channels includes low-threshold Ca^{2+} channels, then in this case, neurons periodically generate not one, but a burst of action potentials (AP). This mode of synchronous activity is normally realized to activate the growth of dendrites and form synaptic contacts between neurons. In the adult brain, the mode is a sign of pathology and appears in epilepsy, ischemic strokes and other brain injuries. The mode has all indications of an autowave: the undamped oscillations, the voltage-dependent of Ca^{2+} channels, the gradient of Ca^{2+} ions as an energy source. Taking into account the autowave nature of calcium oscillations in neurons allows for more intentional control of excitation during the development of the neuronal network, epilepsy and strokes. The experiments were carried out on cultures of various cells. A fluorescent probe Fura-2 was used to detect $[Ca^{2+}]_i$ changes. An inverted microscope Leica DMI6000B was used to register Fura-2 fluorescence.

Conclusions: Taking into account the autowave nature of calcium oscillations in neurons allows for more intentional control of excitation during the development of the neuronal network, epilepsy and strokes.

Thermodynamic and structural characteristics of cryoprotective media for cryopreservation of cells

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Background: Despite the fact that cryopreservation methods are actively developing, when spermatozoa are frozen, cell loss is 40-60%. One of the main causes of cell damage at low temperatures is the formation of extra- and intracellular ice crystals. It is possible to influence the formation of crystals using various temperature regimes, as well as adding special components of cryoprotective media.

Aims: Study of the influence of various components and conditions of freezing on the process of crystal formation in cryoprotective media.

Methods: To obtain thermodynamic parameters, the method of adiabatic calorimetry was used, adapted for the study of liquid media. The study of the process of crystal formation was carried out on the PETRA III synchrotron (DESY, Germany). We studied a water-glycerol solution with a glycerol concentration of 12% by volume with the addition of albumin (4 mg / ml) and sucrose (0.5 M).

Results: In the course of the work, the dependences of the heat capacity on temperature were obtained for an aqueous solution of glycerol (12%), as well as this solution with the addition of albumin and sucrose. It has been shown that the addition of sucrose reduces the area of heterogeneous nucleation, which may contribute to better cell survival. The method of X-ray diffraction analysis was used to study the effect of the components of the medium on the formation of crystals in an aqueous solution of glycerol with a volume of 20 μL during vitrification. It was shown that the addition of sucrose (0.5 M) reduces the size of crystals formed upon freezing a water-glycerol solution, which is consistent with the results obtained by the method of adiabatic calorimetry. Also, the sizes of crystals were estimated for each test solution when freezing in large volumes (1.7 ml) by the method of slow freezing. It has been shown that the average size of crystals in a solution with all components is 8 ± 1 μm. In the course of the experiment, an uneven distribution of crystals within one test tube was found when placed vertically. In this regard, a

horizontal method for placing cryovials was proposed. It is shown that, with such an arrangement, the formation of crystals is statistically significant and occurs more uniformly with a minimum scatter in comparison with the vertical arrangement.

Conclusions: In total, during the work, more than 400 solutions and 30 types of alternative frosts were studied. As a result of the analysis, 2 main methods of freezing were selected as the most effective: horizontal classical freezing, as well as automatic lowering of the sample over nitrogen vapor at a constant speed.

Melatonin effect on oxidative stress dynamics and DNA fragmentation in human spermatozoa

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Background: The imbalance between the production of reactive oxygen species and the cell's ability to absorb them is called oxidative stress. Oxidative stress in spermatozoa leads to lipid peroxidation, DNA fragmentation, changes in motility and, as a consequence, to the loss of the cell's ability to fertilize. The sperm cell is extremely vulnerable to oxidative stress due to the limited amount of cytoplasm and the distribution of enzymes in it. External antioxidants can prevent or slow down oxidative stress. Melatonin is a hormone that is synthesized by the pineal gland, which is able to act as an antioxidant. It has been shown that melatonin is able to prevent lipid peroxidation and oxidation of glutathione in rat brain and liver tissues.

Aims: The aim of the study was to analyze the effect of exogenous melatonin on the development of oxidative stress and DNA fragmentation in human sperm.

Methods:

Oxidative stress directly affects sperm motility rates. Therefore, in the work, the main indicator of measurement is the sperm motility index - a relative value that characterizes the influence of various factors on the ability of sperm to survive. The purified cells were divided into 2 groups (cells with melatonin and cells without melatonin) and incubated for 14 days. The calculation of the mobility index was made relative to the control group of samples, the mobility indices of which were calculated before the start of incubation. Cell counting was performed using a Makler chamber.

To assess the degree of DNA fragmentation, we used the TUNEL method, which serves as a method for direct labeling of DNA breaks. Cell samples were divided into two groups: cells with melatonin (C = 0.5 mM) and cells without added antioxidants. The cells were then incubated at room temperature for 48 h and examined with TUNEL.

Results:

Mobility measurement index: In the first six days, the rate of deterioration in the control sample is significantly higher than in the samples with added antioxidants. After a decrease in the mobility index of a sample with melatonin to 0.85 for 6 days, the cells do not change their state, which is explained by the effect of antioxidants. Melatonin can, according to the literature, act as an interceptor of oxygen radicals.After day 10, all cell groups have an equally low level of motility, which is explained by progressive oxidative stress. On day 12, none of the cells was found alive or mobile in all groups of cells.

TUNEL: If in cells incubated without antioxidants, oxidative processes had already begun after 48 hours (the number of fragmented spermatozoa increased by approximately 6%), then the addition of melatonin slowed down the development of ROS production and DNA fragmentation by almost 2 times (the growth of fragmented spermatozoa was about 3%)

Conclusions: These results underscore the concept of oxidative stress: it can be seen that at the beginning of a long-term experiment, melatonin prevents its development. But then, when the excessive production of reactive oxygen species starts a chain reaction of lipid peroxidation, it is not possible to slow down or stop this process. Also, experiments to assess the degree of

DNA fragmentation allow us to conclude that melatonin can slow down the excessive production of reactive oxygen species in sperm and prevent oxidative stress in the early stages in human sperm, which makes it possible to use it as an antioxidant when working with this type of cells.

Virtual cell and theory of metabolic control.

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Background: During the last decade a huge amount of biochemical information was accumulated due to development of different “omics” approaches in biochemistry (genomic, metabolomic, proteomic, etc). However, we are still very far from an understanding of how multiple metabolic processes in a cell are regulated to provide their coordinated functioning that, in turn, maintains cell homeostasis and physiology.

Aims: Attempts of modern system biology to organize the huge pool of biological date are not very successful. It is generally accepted that one of the main reasons of why mathematics still fails to properly describe the cell biochemistry is a huge scattering of all experimental data in biochemistry, such as enzyme activities, and metabolite concentrations. One source of this scattering is an individual variation of biochemical parameters within a same biological species.

Methods: In this study we used the theory of metabolic control for analysis of metabolism regulation in a “virtual cell” in which all parameters were fixed and, thus, there were no parameter scattering. This virtual cell is a system of differential equations describing glycolysis, metabolism of adenylates, transport Na/K-ATPase, passive transmembrane ion transport, and osmotic volume regulation. The virtual cell was once constructed and successfully used for analysis of effect of glycolysis enzymes deficiencies on viability of human erythrocytes (Martinov M.V., et al., BBA 2000).

Results: Analysis of glycolysis regulation in such virtual cell using the theory of metabolic control recognized different regulatory sites depending on what subsystem of the complete model was considered. For glycolysis only, analyzed separately from other metabolic systems, the theory predicts regulation by hexokinase (HK) and phosphofructokinase (PFK). If subsystem includes glycolysis and ATPase, the ATPase becomes the main regulatory enzyme with a little impact of HK and PFK. And in complete model, including transmembrane transport, the main parameter regulating glycolysis rate becomes a membrane permeability for Na+ and K+. Moreover, the theory of metabolic control cannot predict the actual change in glycolysis rate in response to a change in a regulatory parameter.

Conclusions: This study as well as our experience in mathematical modeling of cell metabolic systems show that an adequate modeling of a metabolic system regulation cannot be achieved without an understanding of its physiological role. Under these circumstances the metabolic control theory has a very limited usefulness.

Topic: Practical applications of cell biophysics: erythrocytes as carriers of drugs

Mathematical modeling of the RBC-bioreactors

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Background: RBC-bioreactors (RBR) are red blood cells in which metabolic pathways are artificially built that are absent in a normal erythrocyte. The built-in metabolic pathway can be used to regulate the concentration of its substrates or products in the patient's blood, which makes RBR potentially useful for the relief of patients with a number of diseases. RBRs utilizing asparagine, ethanol, methanol, ammonium and other substances have been experimentally investigated at different times by scientific groups around the world. However, in most cases, the obtained RBRs showed low efficiency. Since the RBC is a complex metabolic system, the identification of factors limiting the effectiveness of RBR and ways to increase their effectiveness is, in fact, an engineering problem, in the solution of which mathematical modeling of metabolic systems may be useful.

Aims: The purpose of the studies presented in this work was to construct and study mathematical models of previously created RBC-bioreactors in order to identify factors that reduce their efficiency and develop new, more efficient metabolic schemes for creating new RBRs.

Methods: Systems of ordinary differential equations of the first order were used as mathematical models describing the metabolic systems of RBCs with built-in pathways for the consumption of target substrates. In the presented models, in addition to the built-in metabolic pathways, the main parts of the erythrocyte's own metabolism were described (in whole or in part) with which the built-in pathway can interact (glycolysis, pentose phosphate pathway, oxidative metabolism). To study the models obtained, numerical solutions of ODE systems and methods of the theory of dynamical systems were used.

Results: When analyzing the constructed mathematical models of the RBR, two classes of factors were identified that limit their effectiveness. The first group of restrictions is associated with the transport of substrates and products of the built-in metabolic pathway through the erythrocyte membrane. Poor membrane permeability for the substrate leads to a low rate of the target reaction (limited rate of substrate inflow from blood plasma). Poor product permeability can lead to its accumulation inside the erythrocyte and, as a result, cell death from osmotic lysis. The second group of limitations is due to the interaction of the built-in metabolic pathway with the erythrocyte's own metabolic pathways. Such interaction can be carried out through a common metabolite (for example, NAD or NADP) and lead to various effects up to the loss of the steady state of the metabolic system.

Conclusions: Mathematical modeling is a promising method for constructing metabolic systems for potentially effective erythrocytes-bioreactors, which makes it possible to identify and take into account factors that can lead to low efficiency of their work. Despite the wide variety of incorporated metabolic pathways, the number of fundamental limitations is small, and the results obtained in the study of some bioreactors can be used in the design of others.

Development of an automatic device for creating drug-loaded erythrocytes

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Background: Using erythrocytes in drug delivery is a promising technology and is constantly expanding. To standardize the process of incorporating biologically active components (BAC) into erythrocytes automated devices are used. There are only two such devices from Erytech Pharma and Erydel companies around the world that are actively used at the present time. Each of them has its own limitations which reduce the efficiency of the erythrocyte loading process.

Aims: The aim of our work was to create an automated device for the incorporation of BAC into erythrocytes providing a high efficiency of incorporation.

Methods: The device is based on the flow dialysis method using commercial hemodialyzers and build-in automated cell processor for erythrocytes washing. The installation allows using both whole blood and pre-washed erythrocytes as a starting material. The process includes the stages of erythrocytes washing, concentrating the suspension of washed erythrocytes, hypoosmotic lysis of erythrocytes using flow dialysis and subsequent sealing of the obtained erythrocytes containing BAC by restoring the tonicity of the medium. The yield of cells, as well as relative (R) yield of BAC encapsulation were calculated and compared with the corresponding yields of known methods (where R was calculated as the ratio of the enzyme activity in the loaded erythrocytes to the enzyme activity in the initial suspension of erythrocytes)

Results: An automatic device was developed in which all stages of the encapsulation process such as washing and concentrating erythrocytes, dialysis, adding a drug to a suspension, incubating a suspension of erythrocytes are combined into a single device that works without operator intervention using software that controls pumps and valves. We used a standard erythrocyte washing system (APC 215) which was connected to our device with special lines. Distinctive features of this device are the erythrocytes concentration stage before the dialysis procedure, ensuring minimal dilution of the suspension, as well as creating a certain pressure (90-180 mm Hg) in the dialyzer during dialysis and concentrating stages. All this allowed us to obtain a higher efficiency of drug encapsulation relative to the existing analogues of this device. The relative efficiency of the enzyme encapsulation (R) was 51.8 ± 9.6% for L-asparaginase in our developed device, 30.0 ± 3.0% for Na-dexamethasone-21-phosphate (Erydel) and 29.8 ± 2.4% for L-asparaginase using device of Erytech Pharma. The cell yield was very similar for all the studied devices.

Conclusions: A prototype of an automatic device for loading drugs into erythrocytes with a high encapsulation efficiency was created. This high efficiency was reached both due to a selection of the optimal process conditions and due to the presence of concentration stage after erythrocytes washing. Pilot clinical trials of asparaginase-loaded erythrocytes obtained by this device were started.

Topic: Genomics and bioinformatics

Mechanical equilibrium of the cell nucleus and its potential role in regulation of chromatin structure

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Biological functioning of living cells frequently relies on sophisticated cooperation between multiple cellular subsystems that must coexist with each other in a tight and highly crowded cellular space. As a result, the problem of space allocation to each of the cell components plays an important role in intracellular organization. Yet, there is still no full understanding of molecular mechanisms responsible for size regulation of the most of cellular organelles, including even the major ones, such as the cell nucleus. Moreover, despite recent discoveries of the central role of the nucleus geometry in shaping the cell response to environmental cues, molecular mechanisms underlying it remain unclear. To find answers to these questions, we have developed a general theoretical framework aimed at description of DNA packaging in nuclei of living cells, which was used to identify the major molecular processes accountable for the control of the cell nucleus size. Furthermore, the model showed the existence of a previously unknown link between the cell nucleus size and stability of nucleosomes, providing new insights into the potential role of nuclear organization in shaping the cell response to environmental cues.

Topic: Biological systems at supracellular level: hemostasis, immunity and neurobiology

Elevated shear rate is not specific to arterial thrombosis, but also relevant to hemostasis

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Background: Hemostasis is the physiological process leading to the arrest of bleeding. Following vessel wall injury, platelets adhere, become activated and aggregate to form a hemostatic plug. A similar process can take place in a diseased vessel after atherosclerotic plaque rupture and result in the formation of an occlusive thrombus promoting ischemic pathologies such as myocardial infarction or stroke. Blood flow is known to play a central role in hemostasis and thrombosis by regulating every step of this process. The key parameter to define blood flow is the shear rate. The current view is that low flow (100 s^{-1} up to 2,000 s^{-1}) occurs under homeostatic conditions in healthy vessels, while high shear (> 2,000 s^{-1}) is relevant to arterial thrombosis. To date, the blood flow occurring after vessel wall injury and relevant to hemostasis is unknown.

Aims: To evaluate the shear rates at the edge of different types of wounds in mice and humans.

Methods: Intravital and electron microscopy were used to image thrombus formation in several novel models of hemostasis following needle-induced puncture or scissors-induced disruption of various vessels. Two laser doppler probes placed on both sides of the injury measured blood flow in the vessels throughout the hemostatic process, and a ComSol software allowed to recalculate shear rates in the wound. In humans,
the shear rate was calculated by applying Poiseuille's equation with volumetric rates of blood loss after catheter placement.

Results: Intravital microscopy following needle puncture of the carotid artery showed the formation of a platelet- and fibrin-rich hemostatic plug, which was mainly located in the extravascular space. Based on the flows measured experimentally and the size of the injury, we calculated the shear rate at the edge of the wound using Navier-Stokes equations, and found that it was increased up to 100 times as compared to steady state. Additional measures of blood flow performed with a Doppler probe in the femoral vein and the aorta, indicated that the shear rates at the wound were respectively 10 and 33 times higher than the values in the same vessels under homeostasis. Upon scissors-disruption of the spermatic artery, a 3-fold increase at the lesion site was obtained when compared to homeostatic conditions. Finally, in humans, the shear rates in the wound of the cubital vein promoted by a needle resulted in 14,500 ± 5,000 s-1 and 3,700 ± 800 s-1 for 310 and 410 µm of injury diameter, respectively, indicating that the shear reaches high levels following vessel damage.

Conclusions: Our results indicate that high shear rates occur at the edge of various types of wounds in small and large mouse and human vessels. Elevated shear rate is not specific to arterial thrombosis, but also relevant to hemostasis.

Novel insights into the contraction (retraction) of blood clots and thrombi

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Contraction, or retraction, is the spontaneous compression of blood clots by activated platelets. An important functional consequence and the leading structural feature of clot contraction is the compaction and compression deformation of erythrocytes, which take the form of polyhedrons (polyhedra), called polyhedrocytes. The presence of polyhedrocytes in thrombi and thrombotic emboli of different localization indicates that contraction occurs not only *in vitro*, but also *in vivo*.The pathogenetic consequences of thrombus contraction include a decrease in the degree of thrombotic occlusion and a decrease in sensitivity to external (therapeutic) thrombolysis. In the blood of the patients with thrombotic conditions, such as ischemic stroke and venous thrombosis, the contraction of blood clots is inhibited due to chronic hyperactivation and energy depletion of platelets. The degree of the impairment of the contraction of blood clots in thrombotic conditions correlates with the severity of the disease and the risk of thrombus embolization, which indicates the potential diagnostic and prognostic value of a laboratory test for contraction of blood clots as a sign of ongoing or threatening thromboembolism. Based on the available data, the contraction of blood clots and thrombi is an underestimated and poorly understood pathophysiological process, which is of great pathogenetic and clinical significance in thrombosis and pre-thrombotic conditions of various etiologies.

A mechanistic model for thromboelastography

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Background: Thromboelastography (TEG) is a laboratory assay that evaluates the viscoelastic properties of clotting in whole blood samples, and reports a trace representing it. To the best of our knowledge, there are no theoretical models for TEG relating properties of blood to the trace that is produced.

Aims: A mechanistic model for TEG can serve as a useful tool to make predictions for the state of a patient's clotting system

Methods: A mechanistic model is developed for TEG by solving the governing equations for blood flow in an oscillating cup using the semi- inverse approach. The modified shear-thinning Yeleswarapu model is used to represent blood in which the viscosity depends on the shear rate and fibrinogen concentration. Two different cases are considered for the viscosity dependence on fibrinogen concentration: (i) time-averaged fibrinogen concentration dependence, and (ii) instantaneous fibrinogen concentration dependence. The solution of the flow equations yields the velocity variation in space and time. The velocity at the location of the TEG pin is integrated to get the displacement variation with time; the locus of the peaks of the amplitude of displacement is modified using a scaling factor to account for the TEG transducer and obtain the model-predicted TEG tracing.

Results: The model is validated by matching the TEG predictions with real-time TEG data from healthy volunteers, and a good fit is obtained by selecting the transducer scaling factor logically. The model is then used to make predictions for different platelet deficiencies and good match is obtained with clinical data.

Conclusions: The model developed can be a useful start to the development of mechanistic models that relate the properties of blood to the trace produced by TEG, and thus open up a potential tool for predicting coagulopathies.

Corticotropin-releasing factor (CRF) as a mediator of pain response to stressor: involvement of CRF receptors of subtype 2, opioid receptors and glucocorticoid receptors in CRF-induced analgesic effect in rats

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Background: Corticotropin-releasing factor (CRF) is expressed throughout the central nervous system and in peripheral tissues and coordinates a wide range of stress-induced response. Analgesia is one of the characteristics of stress reaction and CRF is involved in providing of stress-induced analgesia (SIA). Exogenous CRF produces analgesic effect in animals and humans. Periaqueductal gray matter of the midbrain (PAGM) plays a key role in generation of SIA. CRF action is mediated by CRF receptors of subtype 1 and 2 (CRF-R1 and CRF-R2 receptors). Both CRF-R1 and CRF-R2 are expressed within PAGM. CRF through CRF-R1 stimulates the ACTH/β endorphin release and, ACTH, in turn, stimulates glucocorticoid production.

Aims: The aim of the study was to investigate the involvement of CRF-R1 and CRF-R2 receptors and opioid and glucocorticoid receptors in analgesic effect caused by central or peripheral CRF administration in conscious rats.

Methods: CRF was administered intra-PAGM (0.7 mkg/rat) or intraperitoneally (40 mkg/kg). The involvement of CRF-R1 and CRF-R2 receptors, opioid receptors and glucocorticoid receptors was studied by antagonists of these receptors: NBI 27914, astressin2 β, naltrexone and RU 38486, respectively. The antagonists were administered centrally or peripherally before CRF injection. Somatic pain sensitivity was evaluated by tail flick latency (tail flick test). The experiments were performed according to the Declaration of Helsinki.

Results: Both peripheral and central CRF administration caused an increase in tail flick latencies (analgesic effect). CRF-induced analgesia was accompanied by an elevation of plasma corticosterone levels. Peripheral administration of NBI 271914 (5 mg/kg, i.p.), astressin2 β (200 mkg/kg, s.c.), naltrexone (1 mg/kg, i.p.) or RU 38486 (20 mg/kg, s.c.) attenuated the analgesia caused by peripheral CRF. In addition, RU 38486 or NBI 271914 by itself led to inhibition of somatic pain sensitivity. Intra-PAGM (1 mkg/rat) administration of naltrexone or astressin2 β attenuated the central as well as peripheral CRF-induced analgesia.

Conclusions: The data obtained suggest that CRF-R1 and CRF-R2, opioid receptors and glucocorticoid receptors are involved in CRF-induced analgesia and one of the mechanisms of CRF-induced analgesic effect on somatic pain sensitivity may be mediated through opioid and CRF-R2 receptors within PAGM. The study was supported by grant of Russian Science Foundation (RSF) № 19-15-00430.

Mechanics of cell interaction, numerical modelling

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Background: The cell is studied numerically, applying the knowledge of mechanical science. The applied knowledge builds on previous work on ultrafine object research. The interaction of the cell is evaluated not only when it is deformed but also when the cell is at a suitable distance from the surface to be interacted.

Aims: In order to understand the behaviour of cells as biological systems, it is important to understand the behaviour of the individual cell. The aim of this work is to describe a theoretical model for analysing the interaction of cell in space and time.
To achieve the aim, the following tasks are considered:

- Formulate and expand the model of oblique interaction of cell, taking into account the motion in normal and tangential directions.

- Introduce and implement a characteristic energy dissipation mechanism related to the deformation of the object, as well as with a change in the effect of adhesion.

- Based on the analysis of ultrafine objects, suggest various interaction models, such as adhesive elastic or adhesive elastic-plastic.

- To implement the obtained interaction models as part of the discrete element method for studying situations of cell movement.

- Numerical experiments and investigation of an oblique interaction of a cell in both normal and tangential directions have been carried out.

Methods: The discrete element method will be used for the numerical experiment. Cell movement is described by acting forces, which are integrated in time using the Gears predictor-corrector time integration scheme. In order to represent the movement of a cell during interaction, the processes of approaching, loading, unloading and detachment are analysed and described.

Results: The results show the interaction of the cell in the normal and tangential directions in the liquid. Using the discrete element method, force, velocity and displacement diagrams are used to represent the results for the interaction process. Due to viscous damping, as well as the different effect of adhesion during bound and rebound process, the interaction of cells has dissipative oscillations over time.

Conclusions: The results show the importance of the influence of fluid on cell behaviour. When applying the adhesive-dissipative model, the cell behaviour has a force-displacement hysteresis. Further research will focus on the rotational behaviour of the cell, taking into account the effect of rolling friction in a fluid on the cell.

Heparin impact on the formation and healing of stomach ulcers in rats

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Background: The incidence of peptic ulcer disease remains at a fairly high level: it is diagnosed annually in 7-14% of the adult population. Despite the fact that in recent years there has been a trend towards a decrease in the number of confirmed cases of peptic ulcer disease, the number of patients with a complicated course of the disease is increasing. One of the pathogenetic factors affecting the development of peptic ulcer disease is a violation of microcirculation. In this regard, it is relevant to study the effect on ulcerative pathology of drugs that affect the rheological properties of blood.

Aims: The aim of the study was to investigate the impact of heparin on the formation and healing of gastric ulcers in experiments on rats.

Methods: The experiments were carried out on the male Sprague-Dawley rats. Gastric ulcers were induced by the application of 60% acetic acid to the gastric mucosa according to the Okabe method (kissing ulcers model) in anesthetized and preliminarily (24 h) starved rats. Four days after the application (day of application - day 0), two “kissing” ulcers were formed in the stomach of the rats, the healing of which, obvious, although incomplete, was observed a week after the application. In this regard, day 4 after acid application was chosen as the time point for ulcer formation, and day 7 for healing. Heparin was administered at a dose of 1000 U / kg (subcutaneously, once a day). When studying the effect on ulcer formation, heparin was administered during the first four days from the day of acid application (0-3 days). In a study of the effect on ulcer healing, heparin was administered during the last three days (4-6 days after acid application) before assessing healing. Control rats were injected with saline instead of heparin.

Results: The introduction of heparin before the formation of ulcers led to a decrease in their area. The average area of formed "kissing" ulcers in the heparin-administered group (32.3 ± 1.8 mm^2) was significantly ($p < 0.05$) smaller than that in the control group with saline administration

(45.8 ± 2.3 mm^2). On the 7th day after the application of the acid, healing of ulcers was observed in both groups, however, in the group with the introduction of heparin, the healing process was accelerated. This is evidenced by a significantly ($p < 0.05$) smaller average area of ulcers in the group of rats with heparin administration (15.8 ± 1.4 mm^2) compared with that in the control group (25.8 ± 1.6 mm^2).

Conclusions: The results obtained indicate the gastroprotective effect of heparin, which is manifested both during the formation and healing of stomach ulcers in rats. This research was supported by the Russian Science Foundation (grant No. 19-15-00430).

Oxidation-induced modifications of the blood coagulation factor XIII (FXIII) at the different stages of its activation identified by mass spectrometry

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Background: Proteins are well known to be among the main targets for reactive oxygen species that can alter proteins structure and their functions. Currently, the studies dealing with oxidative modifications of FXIII are rare in literature. FXIII plays a critical role in supporting coagulation and fibrinolysis due to both covalent crosslinking of fibrin polymers, rendering them resistant to plasmin lysis and crosslinking of fibrin to proteins of fibrinolytic system.

Aims: The present study is aimed at providing evidence that:
the FXIII proenzyme is the least vulnerable target for hypochlorite compared with its activated forms, FXIII partly activated by Ca^{2+} or FXIII fully activated by Ca^{2+}/thrombin.

Methods: FXIII, at different stages of activation, has been oxidized by HOCl/–OCI. Each series of FXIII samples was separated into three portions, which were independently oxidized with 50 or 150 μM HOCl/–OCI and incubated for 1 h at 37° C. The BioVision Colorimetric FXIIIa Activity Assay Kit (BioVision, Milpitas, CA, USA) was used to determine specific transglutaminase activity of activated FXIII before and after oxidation.
The samples were digested with trypsin. Briefly the protein was hydrolyzed with trypsin at an enzyme/protein ratio of 1:50 mixture 16 hours at 37°C. HPLC-MS/MS experiments were carried out on an Agilent 1100 nano-LC (Agilent Technologies Inc., Santa Clara, USA) coupled to a 7T LTQ-FT Ultra (Thermo, Bremen, Germany) high resolution mass spectrometer.
FXIII tryptic peptides were identified by searching the UniProtKB database (UP000005640-9606 HUMAN, Homo sapiens) using PEAKS Studio software (v.8.5, Bioinformatics Solutions Inc., Canada).

Results: The proenzyme treated with 150 μM HOCl/–OCl completely retained its enzymatic activity inherent to the unaffected protein while FXIIIa treated with 50 μM HOCl/–OCl demonstrated the drastically reduced enzymatic activity.
As follows from the mass-spectrometry data, the crucial residues, Tyr560 and Trp279 are covered remaining in native form for all of the samples. However, since FXIII, fully activated by Ca^{2+}/thrombin, dramatically lost activity when exposed to hypochlorite the effect can be driven by damage to amino acid residues in this group of samples, Trp130 and Cys695, as well as Tyr311 and Trp315 located in the immediate vicinity of the catalytic center of the enzyme.

Conclusions: For the first time, the structural and functional damages to FXIII treated with hypochlorite that is recognized the main oxidizer in blood plasma, have been analyzed.

In silico modelling of hemostatic response: challenges and perspectives

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In vertebrates' hemostasis represents a tightly controlled process represented with interwound system of various elements, including cellular and plasma-based reactions. Despite the enormous clinical significance of the principles governing hemostatic response and decades of ongoing research, current understanding of the basic mechanisms that regulate the dynamics of both hemostatic and pathological thrombus formation is very limited. Today in silico approaches became indispensable for investigation of the multiple aspects of the problem that are hardly tackled with the experimental methods.

This overview is aimed to highlight the basic problems in the field and the challenges faced by the current computational models while trying to address multiple burning questions.

To illustrate the principles exploited by the state-of-the art models we give a short overview of the recently described approaches and further provide our view on the existing gaps of knowledge and future instruments required to accomplish these goals.

KED peptide prevents synaptic contacts elimination of hippocampus neurons in an in vivo Alzheimer's disease model

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Background: Cognitive impairment in Alzheimer's disease (AD) correlates with the synaptic contacts elimination. Morphologically, this is manifested in the number decrease of dendritic spines in hippocampal neurons. KED (Lys-Glu-Asp) and EDR (Glu-Asp-Arg) peptides increased the number of dendritic spines in an in vitro AD model.

Aims: The aim of this work is to assess the effect of KED and EDR peptides on the number of dendritic spines in hippocampal neurons in the in vivo AD model.

Methods: For AD model was used a transgenic 5xFAD-M mice cross-line. Animals of this line (3-5 months) were daily injected with KED and EDR peptides (400 μg/kg) or saline (control 2). M line mice, which were injected with saline, served as control 1 (normal).
The density (DS) and the relative number of mushroom (MS) and thin (TS) dendritic spines of CA1 neurons in the hippocampus were estimated on fixed brain slices using confocal microscopy and micrographs analysis in the NeuronStudio software. Statistical analysis of the data was performed using the Statistica 12 software. The animal study was followed the principles of the European convention (Strasbourg, 1986) and the Declaration of International medical association about the humane treatment of animals (Helsinki, 1996).

Results: Normally, DS, MS, and TS were 12.89 ± 0.32 c.u., 44.57 ± 1.11%, and 40.61 ± 1.26%, respectively. In control 2, DS and MS decreased statistically significantly by 15% ($p = 0.011$) to 11.31 ± 0.36 c.u. and by 20% ($p = 0.00002$) to 35.61 ± 1.64%, respectively, compared with the norm. TS increased by 19% ($p = 0.00003$) to 50.15 ± 1.81% compared to the norm.

KED peptide in 5xFAD-M mice statistically significantly increased MS by 16% ($p = 0.024$) to 44.89 ± 1.65% and decreased TS by 13% ($p = 0.008$) to 43.84 ± 1.83% compared to control 2. After KED peptide injection MS and TS did not differ significantly from the norm ($p = 0.157$ and $p = 0.156$, respectively).

EDR peptide administration to 5xFAD-M mice promoted a statistically significant increase in DS by 11% ($p = 0.039$) to 12.64 ± 0.31 c.u. and a decrease in TS by 10% ($p = 0.024$) to 44.84 ± 1.65% compared with control 2.

Conclusions: KED peptide systematic administration prevents the elimination of the most functional synaptic contacts of the mushroom type in the CA1 hippocampus neurons in 5xFAD-M mice. It should be emphasized that the KED peptide normalizes the balance of thin and mushroom spines, which is necessary for memory restoration in AD.

Numerical and qualitative investigation of the mathematical model of blood coagulation with membrane reactions

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Background: Mathematical modeling is extensively used for understanding various aspects of blood clotting. Yet mathematical models remain mostly qualitative and very often contain some non-physiological assumptions.

Aims: The aim of quantitative correspondence of the mathematical models and the experimental results require some corrections in model equations. Set of the model's equations are modified based on systematic bifurcation analysis and qualitative properties. On the other hand, the qualitative correspondence could be achieved by the multi-parametric fitting of the numerical modeling data vs experimental results.

Methods: The set of equations consist of 46 ODE. To solve the ODE system, the one-step Rosenbrock's method with complex coefficients (CROS) was used. By the parameter sensitivity function, we mean the proportionality coefficient between the relative change in the coefficient and the resulting relative change in the function. The sensitivity function is constructed numerically. To achieve the best possible quantitative correspondence, the authors apply the modification of Broyden's method for non-linear optimization. The results obtained clearly demonstrate that the quantitative correspondence requires some changes in the mathematical model.

Results: Introduction of fXI related reaction is proven to be necessary for the mathematical model to avoid non-physiological effect of initial non-zero fIIa concentration. This is a significant qualitative improvement of model as there are no experimental evidence of fIIa existence in blood outside of clotting reaction zones.
Broyden's method is shown to be an efficient tool with automatized capabilities for multiparametric fitting of the experimental data with numerical results.
It can be seen that sensitivity to all test constants occurs when a sufficiently large number of activated platelets and activated V factor are formed in the system. And sensitivity virtually disappears or stabilizes during the period of the fastest increase in thrombin concentration activated.

The effect of the V factor is from platelet activation to the start of the main cascade (about 100 to 400 seconds). In this case, reactions involving factor V play a greater role in the dynamics of the process than the lipid composition of the membrane (sensitivity is higher by about 2.5 orders of magnitude).

Conclusions: The fXI modified clotting model demonstrates qualitative correspondence to the experimental data on fIIa kinetics but also a significant quantitative mismatch. Authors tend to attribute the quantitative discrepancies to the model properties which manifested themselves after removal of masking effect of initial non-zero fIIa concentration. In particular authors suspect that fII activation reactions in the bulk and on platelet membranes are imbalanced after comparing with the experiment.

The role of single nucleotide polymorphisms (SNPs) within genes of platelet glycoprotein receptors and blood-coagulation factors II, V and XIII on coagulation and embolic risk in patients with infective endocarditis

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Background: Despite the progress in the diagnosis and treatment of infective endocarditis (IE) over the past decades, the hospital mortality rate remains consistently high (17.1%-31.3%). Frequent embolic events (EE) make a significant contribution to the structure of mortality. There is an association between various single nucleotide polymorphisms (SNPs) of the genes of the blood coagulation system and the risk of cardiovascular diseases such as myocardial infarction, ischemic stroke. In this regard, the study of the influence of SNP genes of platelet membrane glycoproteins and genes of blood coagulation factors on coagulation and embolic in patients with IE is relevant.

Methods: a prospective cohort study included 81 patients with a reliable diagnosis of IE (according to the DUKE ESC 2009, 2015 criteria). Coagulopathy was present in 52 patients (64%): hypocoagulation in 42 patients (52%) and hypercoagulation in 10 patients (12%). Embolic complications were present in 32 patients (40%). For genotyping, DNA was isolated from EDTA-stabilized peripheral venous blood using the QIAmp DNA Blood Mini Kit (QIAGEN) and the QIAcube automated station (QIAGEN) according to the manufacturer's recommendations. We studied SNPs of 7 genes all of which have been implicated as increasing the risk of arterial tromboembolism and resistance to blood-thinning benefits of aspirin: FII (rs1799963), FV (rs6025), FXIII (rs5985), ITGA2 (rs1126643), ITGB3 (rs5918), GP1BA (rs6065) and GP6 (rs1613662). Genotyping was performed using real-time PCR. Data processing was also carried out using the SNP Stats web program.

Results: No statistically significant differences were identified between the groups with coagulation disorders and EE.

Conclusions: Despite the results, further studies are needed since taking into account the genetic diversity of patients and evaluation of the state of the coagulation system could help identify genetic predictors of embolic complications, develop a strategy for managing patients with high risk of thromboembolism, expand and clarify the indications for surgery, and thus reduce mortality.

Design of potential anti-aggregatory agents and their influence on human platelets activation

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Background: The creation of new anti-aggregatory agents with weak side effects is a fundamental task for bioorganic and medicinal chemistry and medicine in connection with the necessity of the prevention and the treatment of cardio-vascular disorders.

Aims: The main tasks of this investigation included the comparison the anti-agregatory activities of scaffold - 3-(3-pyridyl)isoxazole and two its modifications and the study of compounds series influence on the activation of human platelets.

Methods: The reaction of [3+2]cycloaddition of nitrile oxides to terminal alkynes or alkenes were used for 3-(3-pyridyl)isoxazole and 3-phenylisoxazole derivatives production. The acylation of pyridine-3-carboxamidoxime by benzoylchloride with followed cyclization was used for 3-(3-pyridyl)-1,2,4-oxadiazole derivatives. NMR spectroscopy and mass spectrometry were used. For anti-aggregatory activities determination a “Biola” laser aggregometer (Russia) and washed platelets suspensions were used. The flow cytometry method was used for the study of substances influences on the human platelets activation by thrombin.

Results: A series of 5-substituted 3-pyridylisoxazoles under the Huisgen [3+2]-cycloaddition was prepared and their antiaggregatory activities were studied. The comparison of antiaggregatory activities of 3-(3-pyridyl)-5-phenylisoxazole and 3,5-diphenylisoxazole indicated, that 3-(3-pyridyl)-5-phenylisoxazole was 1.3 times more active. The series of six 3-phenyl-5-pyridylisoxazoles and their 4,5-dihydroderivatives was prepared regioselectively. Anti-aggregatory activities of these compounds are quite high, with IC50 ≤ 100 μmol/L. The replacement of pyridine ring by phenyl ring at position 3 leads to the activity decrease, so 3-(3-pyridyl)isoxazole is more active than 3-phenylisoxazole. The experiments indicate that the activity of 3-(3-pyridyl)-5-phenylisoxazole was 1.1–1.5 times higher than 3-(3-pyridyl)-5-phenyl-1,2,4-oxadiazole. The effect of series of 11 compounds on the human platelet activation with thrombin usage was studied. All compounds suppressed the thrombin induced platelets activation by 11-41%.

Conclusions: According to the experimental data, 3-(3-pyridyl)isoxazole is more active, than two other scaffolds, but all scaffolds may be used for the following design of anti-aggregatory agents. The mechanisms of action of 5-substituted 3-phenylisoxazoles and 3-(3-pyridyl)-1,2,4-oxadiazoles are different than that of 5-substituted 3-(3-pyridyl)isoxazols.
All tested compounds partly inhibited the platelets activation induced by thrombin. The developed procedure for the study of chemical compounds influence on the platelets activation may be used as a test for potential synthetic antiaggregants.
This work was partly supported by Russian Foundation of Basic Research (grant No 17-04-01326a).

Construction of self-adaptive biomaterials surface with high blood compatibility

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Thrombotic and inflammatory complications induced by vascular implants remain a challenge to treat cardiovascular disease due to the lack of self-adaption and functional integrity of implants [1]. The physiological extracellular matrix (ECM) exhibits self-adaptive and self-regulation merits in establishment, separation and maintenance of differentiated tissues and organs. The ECM is three-dimensional, non-cellular structure that is mainly composed of collagens, proteoglycans, glycoproteins, and smaller amounts of other proteins. Biomedical implant mimicking the physiological extracellular matrix (ECM) is a new strategy to modulate the cell microenvironment to improve implant integrity and longevity. Inspired by ECM, we constructed a bio-mimic ECM with a dual-layer nano-architecture on the implant surface to render the surface adaptive to inflammatory stimuli and remodelable possessing long-term anti-inflammatory and anti-thrombotic capability. The inner layer consisted of PCL-PEG-PCL/Au–heparin electrospun fibers encapsulated with indomethacin while the outer layer was composed of polyvinyl alcohol (PVA) and ROS-responsive polymer fibers (PBA). In response to acute inflammation after vascular injury, the outer layer reduced ROS rapidly by PBA degradation for inflammation suppression. The degraded outer layer facilitated inner layer reconstruction with enhanced hemocompatibility through the H-bond between PVA and PCL-PEG-PCL. Moreover, chronic inflammation was effectively depressed with the sustained release of indomethacin from the inner layer. The substantial enhancement of the functional integrity of implants and reduction of thrombotic and inflammatory complications with the self-adaptive ECM were demonstrated both in vitro and in

vivo. Our work paves a new way to develop long-term antithrombotic and anti-inflammatory implants with self-adaption and self-regulation properties [2].

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Acknowledgments:

This work was supported by the financial support of the National Natural Science Foundation of China (52061135202, 51573186, 21807097), National Key Research and Development Program of China (2018YFE0121400, 2016YFC1100402).

The influence of blood clotting on pulmonary circulation

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Background: During the coronavirus disease, multiple blood clots can form in the lung arteries due to thrombo-inflammation initiated by viral infection, and possibly leading to a life-threatening decrease in blood oxygenation. In this work, we develop a new model describing the mutual influence of thrombus formation and blood flow in the vascular network in order to evaluate the decrease in pulmonary circulation depending on the level of vascular obstruction. The proposed approach can be used to study the effect of clotting on blood flow in other complex vascular networks.

Methods: Reaction-diffusion equation for the thrombin concentration is used to describe clot growth in flow. Analytical estimates of the maximal clot size and conditions of vessel occlusion are validated by the comparison with numerical simulations of the Navier-Stokes equations in a two-dimensional domain solved with the Computational Fluid Dynamics library OpenFOAM [1]. The graph of pulmonary vessels is constructed using the Cardio-Vascular Simulation System (CVSS) [2], where the flow velocity and pressure are modeled with the equations of 1D hemodynamics. These equations are coupled with the model of thrombin concentration in order to describe the influence of clot growth on blood circulation [3].

Results: The model describing blood circulation in a complex network is developed taking into account clot growth in inflamed vessels. The level of vessel obstruction and the reduction of total flux in the network are estimated depending on the parameters of the coagulation cascade influenced by the inflammation.

Conclusions: Even a small lung obstruction due to thrombo-inflammation can lead to an essential flux reduction. As such, 5% of network volume reduction due to clotting can cause up to 12% of flux reduction and the corresponding drop of blood oxygenation.

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Modelling of blood clotting in aneurysm

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Aneurysms of saccular shape are usually associated with a slow, almost stagnant blood flow, as well as a consequent emergence of blood clots. Despite the practical importance, there is a lack of computational models that could combine platelet aggregation, precise biorheology and blood plasma coagulation into one efficient framework. In the present study we address both physical and biochemical effects during the thrombosis in aneurysms and blood recirculation zones. We use continuum description of the system and partial differential equation-based model that accounts for fluid dynamics, platelet transport, adhesion and aggregation, and biochemical cascades of plasma coagulation. The numerical simulations indicate an important role of RBCs in spatial propagation and temporal dynamics of the aneurysmal thrombus growth.

Simulation of the coagulation system: Tool or Toy?

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Several investigators of high reputation prractice *in silico* simulation of the clotting system.

We have done this ourselves in an early stage, but then asked ourselves the question of in how far simulation can help in understanding global tests of the coagulation system such as the thrombin generation test.

The essential objection is that there are many ways in which an extensive set of differential equations, such as required to represent the complicated clotting system (up tp about one hundred) requires up to a hundred parameters to obtain an output, i.e. the TG-curve, characterised by four parameters.

We demonstrated that an unrealistically reduced mechanism of thrombin generation, lacking the haemophilic factors, can perfectly simulate the effect of infusion of a haemophilic facyot on thrombin generation in a haemophilic. This proves that proposing mechanisms, translating them into thrombin generation curves and drawing conclusions on the outcome is a dead end.

We approached the question from the other side, and found the minimal set of ordinary differential equations that results in a thrombin generation curve that cannot be distinguished from an experimentally obtained one. It appered to need only six input parameters. We then found the corresponding chemical reaction mechanism.

It appeared that even six parameters can be varied in an infinite number of ways to obtain the same output (TG-curve).

We conclude that simulation is an intellectual toy of high standing but should not be considered a reflection of (patho-) physiological reality. Notably it cannot be used to understand diseased states and represents a real danger to the patient when it is.

Spatial effects in the complement system

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Most biological systems have positive feedback loops in their structure. One of them is the human complement system (CS). It consists of about 40 different proteins. One part of them is located on organism cell membranes, while the others are soluble, nevertheless cell membranes are needed for the system activation [1]. The main purpose of their work is to destroy and to eliminate pathogens from the host organism. They can assemble a membrane-attack-complex (MAC), thus the foreign particle lysis takes place [2]. The central protein of the cascade is C3. There is an amplification loop which gives the system an opportunity for nonlinear behaviour [3]. We suppose that this can be the main cause of some CS diseases development.

The main CS diseases are connected with different activation inhibitors deficiencies [4]. Most patients' diseases are due to genetic defects of these factors. However, these diseases don't usually manifest at an early age and they're usually associated with different viruses and bacterias [5].

Thus, our assumption is that pathogen can be a trigger, its appearance provokes the CS activation, there will be a lot of active molecules. New molecules won't have enough space on a membrane after a while. They will be able to activate a loop in a peri-membrane space. New active molecules will diffuse and activate new proteins contributing to active protein autowave spread in space.

In our study healthy human erythrocytes were attached to a flow chamber surface and were pre-opsonized. The different concentrations of autologous serum were pumped through the chamber. Our special program was able to simulate a lysis in a chamber. There is a special parameter which is responsible for the autowave spread. We counted lysed erythrocytes in the chamber that had the dead neighbours and compared it with program data. Experiments show that the percent of dead cells was higher than in the model without the spread. Thus, we think that having a dead neighbour increases the probability of a cell's death.

Experimental data indicate that there is a spatial transfer of active forms of proteins in space and it possibly can influence the development of various CS diseases.

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Synergism between Anticoagulation and Phototherapy to Prevent the Thrombotic Complications

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Cancer patients face risks of a broad range of thromboembolic complications, which threaten their lives. Some procoagulant substances, secreted by cancer cells, diffuse into bloodstream and lead to the hypercoagulable state of cancer patients. What's more, the tumor hypoxia also can increase the thrombosis risks due to the down-regulation of S protein, an inhibitor of IXa factor. Besides, the traditional cancer treatment has been proved to increase the probability of venous thrombosis (VTE), such as surgery, chemotherapy and radiotherapy, which induces the vascular injuries and activates the coagulation pathway. Chemotherapy also can lead to the hypercoagulability through other mechanisms, such as reduction of natural coagulation inhibitors such as C protein, S protein and antithrombin III and activation to platelets. Therefore, appropriate clinical anticoagulant intervention is suggested in cancer patients.

Topic: Methodological issues in biophysics

How does viscosity help (!) the dynamics of spatial self-organization of molecular and supramolecular structures?

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Background: All of important molecular and supramolecular biological processes occur in viscous media. The friction forces for a particle moving in a viscous medium are proportional to the viscosity and lead to the dissipation of the energy of motion into heat. However, viscosity has one more important function, which is discussed in the report. First, we consider an example of the molecular dynamics of folding of a polypeptide chain of 150 residues in a medium with a viscosity of the order of the viscosity of liquid water. The polypeptide chain folds into a helical conformation and then forms one of the characteristic folds found in proteins. A more careful correlation analysis of the motion of the polypeptide chain shows that the phi-type angles turn predominantly in one direction, and the psi-type angles - in the opposite side. Modeling the dynamics of the same system in a medium with a viscosity below the critical value demonstrates the absence of correlations between rotations of torsion angles and the macromolecule folds into a stochastic globule. This example demonstrates the "organizing" effect of viscosity on the dynamics of a system of a large number of interacting particles.

Aims: In the report, we analyze the dynamic properties of such systems from the first principles of mechanics and show that the dynamics of a system of a large number of interacting particles in a viscous medium obeys certain statistical laws. The more degrees of freedom are involved in the process the more accurately the system obeys these laws [1]. We use simple and intuitive geometric properties of multidimensional spheres and a theorem on the practically constant value of a good physical function of a large number of variables on the hypersphere surface of a finite radius (the cognitive dissonance of such a statement can be reduced by a simple argument - a volume bounded by a hypersphere of a finite radius approaches 0 for a large number of dimensions of the hypersphere, i.e. the hypersphere "tends" to a point).

Methods: The analysis of the dynamics of a system of a large number of particles in a viscous medium, carried out from the first principles of mechanics, shows that under practically significant conditions, several important principles for motion are implemented, which strongly influence the formation of the spatial structure of a system of particles during spontaneous folding.

Results: These principles are as follows:

- The average rate of energy dissipation is uniformly distributed over all degrees of freedom.
- The movement occurs in such a way that the conditions of the maximum possible rate of decrease in the potential energy of the total system and the minimum possible rate of energy dissipation are simultaneously fulfilled.

The last statement may seem contradictory, since these values are equal to each other. But it should be taken into account that we are considering motion in a phase space of high dimension and these extreme principles work in different areas of this space. To illustrate these principles, the report presents the results of molecular modeling.

Conclusions: The above principles for the motion of a system of a large number of particles in a viscous medium are especially interesting for understanding the regularities of the spontaneous formation of spatial structures of linear biopolymers in a viscous (aqueous) medium. The movement of a representative point along the multidimensional energy landscape occurs along most smooth paths, avoiding sharp drops in potential energy over a relatively small number of degrees of freedom. This reduces the probability of a representative point falling into "energy traps" on the way to the global minimum energy.

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Euler arches and Duffing oscillators a few nanometers in size

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Background: Bistable macromolecules are attracting increasing attention due to the intensive design of a wide range of nanodevices acting as two-state machines.

Aims: How small could a machine be? We attempted to shed light on this question using molecular dynamic simulations of short oligomers subjected to force loads.

Methods: The oligomers and environmental water were modeled in a fully atomistic representation with a time step of 2 fs using Gromacs 2019.15. The OPLS-AA16 force field for the oligomer and SPC/E model for water were used. The temperature was set at 280 K by velocity-rescale thermostat. The effective length of the trajectories were about microseconds (for more details, see [1,2]).

Results: The results turned out to be more impressive than we had expected. We found that short N-isopropylmethacrylamide oligomers and short pyridine-furan springs, themselves only a few nanometers in size, reacted to force loads in a manner similar to bistable mechanical systems such as Euler arches and Duffing oscillators, respectively [1-3]. Besides deterministic transitions between the two states controlled by external forces, the noisy induced spontaneous vibrations of the bistable oligomers were observed near the critical load. In our simulations, the thermal bath was the only source of the noise. We also demonstrated the so-called stochastic resonance for both oligomers. We showed that weak oscillating forces applied to the oligomers transform the spontaneous vibrations into almost regular but still noisy-induced switching between the two states.

Conclusions: Thus, our simulations show that particular oligomers and/or oligomeric compositions, even if they are only a few nanometers in size, can exhibit the dynamics characteristic of bistable machines. Bistability barriers of the oligomers we have specified are reasonably high to separate two states of the oligomers against the thermal noise. On the other hand, just the thermal noise activates the transitions between the two states. Our simulations give some reasons to believe that this may be the case, but the proof requires challenging experimentations.

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Platelet hemostasis reactions are tightly regulated by temperature

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Background: Platelet shape change, aggregation and clot formation, the key reactions of cellular hemostasis, are well distinguished and registered by the laser diffraction method using a recently developed new device (LaSca-TMF) which allows detection kinetics of platelet transformation together with changes of intracellular calcium (Ca_i).

Aims: We presented data on how reactions of cellular hemostasis are regulated by the changes in the temperature from 25^{o}C to 41^{o}C.

Methods: Blood was obtained from healthy volunteers in accordance with the Declaration of Helsinki. For Ca_i determination, platelet-rich plasma (PRP) was incubated with Fluo-3 AM, then PRP was diluted 20-fold by HEPES buffer containing 2 mM Ca^{2+} and platelets were activated by ADP.

Results: Shape change. The velocity of the reaction significantly increased with the temperature (τ1/2=15.9±3.1s at 25°C; 6.1±0.7s at 37°C; 4.6±0.4s at 41°C). However, EC50 for ADP did not change significantly (EC50=40.9±9.9nM at 25oC; 46.9±9.1nM at 37°C; 49.4±8.6nM at 41°C).

Aggregation. ADP at low doses (80 ÷ 300 nM) induced aggregation at 25oC, whereas the aggregation at 37 and 41oC required higher ADP concentration (>600nM). The EC50 at 25°C was 96±16nM, at 37°C was 594±76nM, and at 41°C was 808±61nM.
Clot formation. Continuous stirring of PRP (1200 rpm) during 9-15 min initiated spontaneous clot formation which was decreased by an increase of temperature from 25 to 37°C. Spontaneous clotting time (min) at 25°C was 14.7 ± 0.4, at 37oC was 11.9 ±1.3, and at 41°C was 11.8 ±1.0. Importantly, ADP even at a high concentration (2 μM) did not significantly influence clot formation reaction.

Regulation of Intracellular calcium concentration. Two opposite intracellular mechanisms are responsible for the regulation of Cai concentration in platelets. The fast increase of Cai is mediated by the release of calcium from intracellular stores and influx through the plasma membrane. Calcium efflux is slower and mediated by the activity of Ca^{2+} ATPases. To characterize the dynamic of changes in Cai induced by ADP we calculated the area under the curve (AUC) during the first 20 sec following ADP administration. Surprisingly we found that increase in temperature significantly accelerated calcium efflux. EC50 for ADP at 25oC was 603±28 nM, at 37°C was 939±39 nM, and at 41°C was 1156±99 nM.

Conclusions: Presented data demonstrated that key reactions of cellular hemostasis are dependent and differentially regulated by the temperature. Under hypothermic conditions the velocity of shape change reaction and clot formation decreased, whereas aggregation is accelerated at low (<600nM) doses of ADP. One of the explanations concerning accelerated aggregation under hypothermic conditions might be connected with slow efflux of Ca_i at 25°C. Prolonged high Cai could trigger intracellular reactions of signal transduction that leads to platelet activation.

Reversible aggregation and deformation of RBC as determining factors of blood fluidity control

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Background: Red blood cells (RBC) microrheologic properties (MP) strongly influence blood fluidity and, consequently, the microcirculation of blood and human health in general. These RBC intrinsic properties including their reversible aggregation and deformation in shear flow, in particular, in microcapillaries, change in various pathologies, e.g., due to elevated binding of fibrinogen, the major aggregation-inducing molecule in blood plasma, to RBC membrane. Changes in RBC MP can take place aiming to control blood fluidity endogenously by the organism itself or exogenously via medications.

Aims: This work aims to characterize the RBC aggregation, deformability, capillary flow and fibrinogen-membrane binding properties using in vitro and in vivo optical measurements.

Methods: We used RBC aggregometry method based on diffuse light scattering from whole blood samples in microfluidic flow chambers, laser diffractometry technique from dilute suspensions of RBC in controlled shear flow, laser trapping and manipulation of single RBC, and fluorescence microscopy and flow cytometry to assess the mechanisms of fibrinogen interaction with RBC membrane and possible ways to control it (all in vitro). These measurements were performed with blood samples freshly drawn from cubital veins of healthy

volunteers or patients suffering from various diseases following their informed consent. The blood samples were always stabilized in order to prevent clotting. Also, we used digital capillaroscopy of blood flows in nail bed capillaries in vivo.

Results: The results of large series of measurements based on a group of healthy volunteers and several groups of patients suffering from arterial hypertension and type 2 diabetes mellitus show significant impairment of MP, in particular, enhancement of RBC aggregation both in large populations of cells and in cell doublets. Statistically significant correlation of these changes with alterations of blood capillary flow parameters was found, which indicates the control effect of the MP on blood fluidity. Our results on the effects of fibrinogen-binding inhibition, show the specific character of the adsorption of fibrinogen on RBC membrane. This indicates the existence of a fibrinogen receptor on the surface of RBC membrane, which may be a glycoprotein receptor class IIb/IIIa. This result potentially presents novel opportunities to control the rate of fibrinogen adsorption on RBC membrane and, therefore, correct the MP disorders caused by elevated fibrinogen-induced RBC aggregation.

Conclusions: Optical characterization of RBC microrheology and blood microcirculation performed using in vitro and in vivo measurements enables one to determine significant alterations in important features of human organisms that may need urgent clinically corrections.

Acknowledgments: The study was supported by RFBR (grant No19-52-51015).

Oxidative modification of fibrinogen molecules: structural and functional aspects

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Background: Human fibrinogen, a key protein in plasma hemostasis, is a highly vulnerable target for oxidants. Fibrinogen undergoes posttranslational modifications that can potentially disrupt the structure and function of the protein.

Aims: The study was aimed at both the search for oxidative modifications within the fibrinogen molecule subjected to HOCl/ − OCl-induced oxidation and the effect of oxidation on the structure and function of fibrinogen.

Methods: Fibrinogen was purified from the plasma by glycine precipitation. Fibrinogen was oxidized by HOCl/ − OCl. Post-translational modifications were detected by HPLC-MS/MS. The effect of oxidation on gelation was studied by elastic light scattering (ELS) and confocal laser scanning microscopy (CLSM), monitoring the kinetics of fibrin gel formation and a number of biochemical methods.

Results: Amino acid residues located on all three chains of the protein were revealed to be involved in oxidation. The αC-connector was shown to be most vulnerable to oxidation as compared to other structural parts. The E region turned out to be the most protected area of the protein. Numerous amino acid residues responsible for the conversion of fibrinogen to fibrin remained unaffected upon the fibrinogen oxidation. As evidenced by ELS and CLSM-obtained fibrin morphology images HOCl/–OCl caused the formation of abnormal fibrin with a decreased diameter of individual fibres.

Conclusions: - Amino acid residues located on all three chains and main structural parts of the protein were revealed to be involved in oxidation.

-The αC-connector was shown to be most susceptible to oxidation.

-The E region proved to be least vulnerable to the action of the oxidant.

- Amino acid residues responsible for the conversion of fibrinogen to fibrin remain unaffected upon fibrinogen oxidation.

- Treatment of fibrinogen with HOCl/ − OCl promoted a dose-dependent damage to the fibrin structure.

- Some of Met residues in the fibrinogen structure could serve as ROS scavengers.
The research was done on the confocal microscope Zeiss Axio Observer Z1 of the Collective Use Center "Modern methods of experimental biophysics"; of the Center for Theoretical Problems of Physicochemical Pharmacology of the Russian Academy of Sciences. The study was supported by the Russian Science Foundation (№21-74-00146).

Development of a track-etched membrane based method of the isolation of CTCs

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Background: It is believed that about 90% of deaths occur from metastasis, when tumor cells separate from the primary tumor, enter the bloodstream and lymphatic system, spread throughout the body, giving rise to secondary tumors.1 Such cells are called circulating cells (CTCs). For the last few years, a large number of techniques for CTC`s detection and analysis has been implemented. These techniques rely mainly on tissue specific markers expression of tumor cells or tumor cells physical properties that are different from those of normal blood cells, some methods utilize the combination of approaches.

The main purpose of this work is to develop a track-etched membrane-based method of the isolation of CTCs ready for further processing.

Methods: MCF7, MDAMB-231, and T47D breast cancer cell lines were a gift from National Medical Research Radiological Centre of the Ministry of Health of the Russian Federation. Based on average CTC size (approx. 16 um (LINK)) commercial track-etched polycarbonate membranes (it4ip, Belgium) with minimal fraction of coupled pores with the following membrane parameters were used: 1) 6,5 um pore size, 1% of porosity, 96.1% of single pores and 2) 7 um pore size, 1.2% porosity 95,5 % of single pores. Cells were carried in constant contact with the liquid media to achieve better cells viability.

Results: Filtration protocol was tested on model system with cells of a breast cancer cell lines introduced into buffer or healthy donor`s blood. As shown on figure 1, the sensitivity (The ratio of to the number of cells found on a filter to the number of cells introduced into the model system.) is approximately 0.9. The level of cells integrity similar to smear was achieved (figure 2,3). Further cells were put to IGC cytokeratin and EpCAM staining.

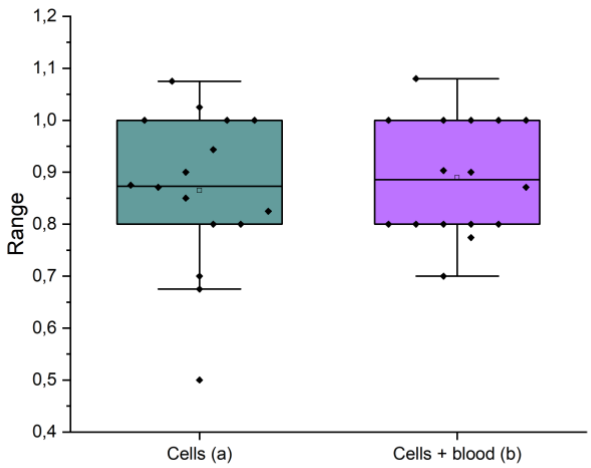

Figure 1. The sensitivity of the method with model systems: a) cells suspended in buffer, b) cells suspended in healthy donors blood

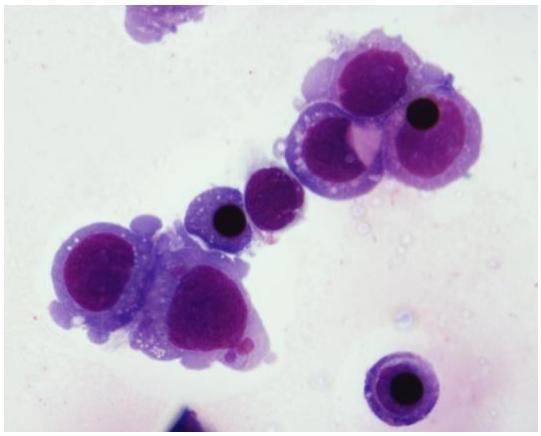

Figure 2. MCF7 cells filtered from blood 100x.

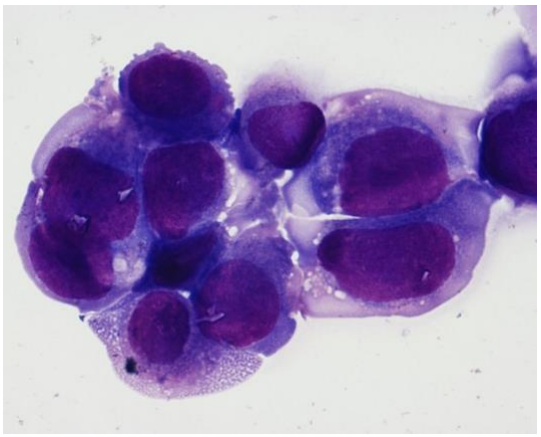

Figure 3. Smear of MCF7 cells on glass 100x.

Conclusions: Constant contact with liquid medium is important to maintain cells viability. Given method allows to extract living cells ready for further processing, i.e. cultivation, immunofluorescent staining etc.

Topic: Methodological issues in systems biology and systems physiology

A possible mechanism of ligand-receptor binding of short peptides to NaV1.8 channels: a novel approach to development of safe and effective analgesics

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The effects of short peptides on NaV1.8 channel voltage sensitivity were studied by the patch-clamp method in the “whole-cell recording” configuration and estimated basing on evaluation of the effective charge (Zeff) of the NaV1.8 channel activation gating device. Experiments were performed on dissociated sensory neurons isolated from the L5-S1 region of the spinal cord of newborn Wistar rats, as published elsewhere [1]. Conformational analysis of the studied peptides was carried out by Monte Carlo method with energy minimization [2].

Two peptides, Ac-RER-NH2 (1 μM) and Ac-RERR-NH2 (100 nM), significantly decreased the Zeff value, while Ac-RAR-NH2, Ac-REAR-NH2, and Ac-REAAR-NH2 did not exhibit this effect. The distance between the guanidinium moieties in the molecules of the former two peptides was found to be in the range from 10 to 14 A.

The NaV1.8 channel activation gating device is shown to be directly modulated by several arginine-containing peptides. Arginine residues play the key role in ligand-receptor binding of short peptides to the NaV1.8 channel due to formation of intermolecular ion-ionic bonds involving positively charged guanidinium moieties. The guanidinium moieties should be positioned at a specific distance from each other to provide effective ligand-receptor binding.

NaV1.8 channels are responsible for the nociceptive signal coding. Selective modulators of their functioning can have a therapeutic application. The most pronounced decrease of Zeff was produced by Ac-RERR-NH2 at the concentration (100 nM) corresponding to those of comenic acid and endogenous ouabain, which indirectly modulate the functioning of NaV1.8 channels [3, 4]. All these three structurally diverse agents are the promising candidates for the role of a novel safe analgesic that can replace opioids and opiates in clinical practice.

The study was supported by the Ministry of Science and Higher Education of the Russian Federation under the agreement № 075-1502020-919 from 16.11.2020.

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Microgravity-induced bone loss: lessons learned from systematic reviews and meta-analysis

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Background: Space exploration remains technologically and medically challenging. While large-scale medical studies are impossible in space travelers, meta-analysis allows combining data from small crews that participated in space missions over several decades.

Aims: To quantify the spatial and temporal aspects of microgravity-induced bone loss, we performed a meta-analysis of systematically identified studies.

Methods: We searched Medline, Embase, Web of Science, BIOSIS, NASA Technical reports, and HathiTrust, with the last update in November 2019. From 25 articles selected to minimize the overlap between reported populations, we extracted post-flight bone density values for 148

individuals, and in-flight and post-flight biochemical bone marker values for 124 individuals who participated in spaceflights ranging from 4 to 252 days in duration.

Results: The reported sample size varied from 1 to 58 people, however limited availability of individual parameters did not allow stratification even by age, sex, and mission duration. Overall, a percentage difference in bone density relative to pre-flight was positive in the skull, +2.2% [95% confidence interval: +1.1, +3.3]; neutral in the thorax/upper limbs, -0.7% [-1.3, -0.2]; and negative in the lumbar spine/pelvis, -6.2 [-6.7, -5.6], and lower limbs, -5.4% [-6.0, -4.9]. In the lower limb region, the rate of bone loss was -0.8% [-1.1, -0.5] per month. Bone resorption increased hyperbolically with a time to half-max of 11 days [9, 13] and plateaued at 113% [108, 117] above pre-flight. Bone formation remained unchanged during the first 30 days and increased thereafter at 7% [5, 10] per month. Upon landing, resorption markers decreased to pre-flight levels at an exponential rate that was faster after longer flights, while formation markers increased linearly at 84% [39, 129] per month for 3-5 months post-flight.

Conclusions: These estimates allowed us to calculate sample sizes required to detect spaceflight-related changes in bone parameters, thus informing the design of future spaceflight studies. Understanding the dynamics of changes in bone density and turnover in space travelers is imperative for a design of successful countermeasures.

Reimagining the interaction between stress-induced glucocorticoids and gastric ulceration: the importance of a methodological approach

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Background: Activation of the hypothalamic-pituitary-adrenocortical (HPA) axis is a main distinctive feature of stress reaction. One of stress-related pathology is a form of gastric ulceration, called “stress ulcer”. Hans Selye attracted an attention to relation between stress-induced glucocorticoids and gastric ulcer when he described adrenal hypertrophy and gastric ulceration as the symptoms of a triad of stress. For several decades it has been generally accepted that glucocorticoids produced during stress are ulcerogenic hormones.

Aims: As this widely accepted view about ulcerogenic role of glucocorticoids produced during stress contradicts their adaptive role, we further clarified the question. Our work hypothesis was that stress-produced glucocorticoids are gastroprotective.

Methods: To verify the hypothesis, the effects of deficiency of stress-produced glucocorticoids with subsequent corticosterone replacement as well as the effects of blockade of glucocorticoid receptors on stress-induced gastric ulceration were studied in rats. To inhibit corticosterone production during severe stress we used several various methodological approaches: elimination of the hypothalamic corticotropin-releasing factor (CRF)-producing neurons, inhibition of CRF synthesis in hypothalamus, blockade of CRF receptor type 1, ACTH immunoneutralization, inhibition of corticosterone synthesis. Various types of immobilization in combination with low temperature were used as ulcerogenic stress stimuli in preliminary fasting rats. The experiments were performed according to the Declaration of Helsinki.

Results: We found that a reduction in corticosterone release in response to ulcerogenic stress aggravates gastric erosion produced by this stress. Pre-treatment rats with of glucocorticoid receptor antagonist RU-38486 also resulted in significant aggravation of stress-caused gastric injury. Moreover, we demonstrated that in rats with inhibited stress-induced corticosterone rise normally non-ulcerogenic stimuli are transformed into ulcerogenic ones. Additionally, our findings argue for the contribution of glucocorticoids to protective influence of preconditioning mild stress against stress-induced gastric injury.

Conclusions: The results of our experimental studies prove the adaptive, gastroprotective, nature of glucocorticoids produced during acute stress-caused activation of the HPA axis. The study was supported by grant of Russian Science Foundation (RSF) № 19-15-00430.

Application of machine learning technologies in a non-invasive sensor system for diagnostics of a human functional state

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Background: For the successful solution of the issues of assessing the performance of specialists in various professions, the availability of quick, regular and mass diagnostics of physical health and functional state is especially important. In the context of organizing diagnostics outside medical institutions, with limited access to high-resolution diagnostic equipment (tomographs, etc.), relatively cheap high-speed diagnostic complexes of a new generation are used, based on the introduction of modern biomedical methods of health control, determined by key physiological and biochemical parameters of the respiratory and cardiovascular systems.

Aims: The aim of the study is to develop an automated complex with an optical sensor analyzer for determining the oxygen status of human tissues by the content of the main fractions of hemoglobin, which makes it possible to form a "digital image" of the oxygen status of a subject's tissues and its subsequent recognition during the diagnostic process.

Methods: A diagnostic system for analyzing the oxygen status of human tissues has been developed and investigated, which consists of two modules: optoelectronic and computational information. The analyzer is based on the concept of the processes of absorption and scattering of light directed at tissues, and the amount of backscattered radiation, which depends on the penetration depth of light quanta and their maximum absorption by their components. The essence of the method consists in recording the spectral intensities of the scattered part of the light returning back to the skin surface and outward, where the photodetector registers its spectral intensities, which change after optical processes in tissues, depending on the presence of certain substances in the investigated area of the body.

Results: Experimental studies with the participation of 31 male subjects aged 18 to 23 years were carried out in order to obtain measurements of a multisensor diagnostic optical system, which are a combination of the numerical readings of six optical sensors in arbitrary units. The diagrams obtained in the process of data processing are “digital images” of the oxygen status of the subjects, reflecting the status of oxygen supply to tissues and the general functional state of the body. As a result of their assessment, it was shown that each subject has his own individual and unique "image". Cluster analysis of multidimensional data was carried out using artificial neural networks in the form of self-organizing Kohonen maps and the method of principal components. The multivariate data were the numerical readings of each of the six sensors after the functional load of 31 subjects. A comparative analysis of the results of calculations was made in the course of data processing by various methods. Was built a two-dimensional lattice 3 x 3 (31 subjects "self-organize" on 9 neurons (nodes) of the output layer of the Kohonen network.

Conclusions: The results obtained represent groups of subjects with different compensatory-adaptive responses to the load. The division of the subjects into groups correlates with independent biomedical studies reflecting the real compensatory-adaptive responses of the subjects. In addition, according to a medical and biological study, two subjects who did not fall into any of the groups, at the time of the experiment, had an unfavorable compensatory-adaptive reaction and an unsatisfactory functional state of the body. The research results indicate the effectiveness of the created diagnostic complex and the prospects for studying the oxygen status of human tissues using the methods of optical multisensor systems in order to assess adaptive responses, functional state and performance of specialists of various professions.

PI3 kinase ligands LY294002, LY303511, and PI828 block coupling of the 5-HT2C receptor with PI3 kinase-independent mobilization of Ca^{2+}

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Many GPCR receptors are associated with the mobilization of intracellular Ca^{2+}, which is a regulator of many cellular functions and intracellular processes from gene expression to apoptosis. Intracellular Ca^{2+} signaling is regulated by a number of enzymes, including class I phosphatidylinositol 3-kinase (PI3-kinase).

Here we studied Ca^{2+} signaling in single cells of the monoclonal CHO-cells expressing the recombinant 5-HT2C receptor, and analyzed the possible role of PI3 kinase in the generation of serotonin-induced Ca^{2+} signals. Short-term stimulation of cells with serotonin-induced pulse Ca^{2+} responses in them, which were blocked in the presence of PI3-kinase inhibitors LY294002 (5 μM) and PI828 (3 μM). It turned out that these compounds had an inhibitory effect when applied simultaneously with the agonist, although inhibition of intracellular targets usually takes time for the inhibitor to penetrate the plasma membrane and accumulate in the cytosol at a sufficient level. At the same time, LY303511 (5 μM), which is an analogue of LY294002, inactive with respect to PI3 kinase, also blocked cell responses to serotonin, both under conditions of pre-incubation and when applied simultaneously with an agonist. The listed inhibitors differ little from each other structurally, while the PI3-kinase inhibitor of another chemical nature - wortmannin (10 μM) - did not affect the ability of cells to generate Ca^{2+} responses to serotonin.

Taken together, these facts indicated that the effects of LY294002 and PI828 were not associated with inhibition of PI3 kinase, but were most likely mediated by extracellular mechanisms, including a direct effect on the serotonin receptor.

It should be noted that a number of PI3 kinase inhibitors are used as antineoplastic drugs that have a wide range of side effects. It is possible that the ability of substances of this class discovered by us to antagonize the 5-HT2C serotonin receptor may underlie some of them.

This work was supported by the Russian Science Foundation grant 20-74-00056.

Processes in photosynthetic membrane as an object of systems biology

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Background: The processes in the photosynthetic membrane ensure the existence of life on Earth. In photosynthis the absorbed quanta of solar energy are converted into energy of electric and electrochemical potential, and then into the energy of chemical bonds. This complex system is a subject of numerous experimental and theoretical investigations.

Aims: Processes in photosynthetic membrane are studied experimentally at the level of individual molecules (Chl, PQ, Cyt), molecular complexes (PSI, PSII), subcellular structures (thylakoid). In contrast to other biological systems, the values of the elementary reaction constants of photosynthetic electron transport and coupled reactions are estimated using the spectral methods. Our aim was to develop mathematical models to simulate the processes in phorosynthetic membrane at molecular and cellular level.

Methods: To simulate the data of fluorescent and other spectral experimental methods we developed kinetic models using differential equations and Monte Carlo approach on the time scale mikroseconds-seconds. To simulate the photosynthetic protein interactions in the interior of photosynthetic membrane we developed Multiparticle Brownian models. By Molecular

dynamics models we simulate step-by-step complex formation mechanisms during photosynthetic electron transport on a time scale from picoseconds to hundreds of milliseconds.

Results: Kinetic models of photosynthetic electron transport of different degree of detail help to interprete spectral experimental data on the state of photosynthetic system at normal and stress conditions. Agent-based (molecular, Brownian, Monte Carlo) models clarify biophysical mechanisms of photosynthetic chain component interactions.

Conclusions: Various methods of mathematical modeling developed on the bases of experimental data elucidate the mechanisms of primary photosynthetic processes at the level of molecules, molecular complexes and photosynthetic membrane. General results are used in biotechnology and environmental monitoring to evaluate the state of photosnthetic apparatus at different stress conditions.

Quantum docking

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Background: It is difficult to overestimate the importance of docking for the development of inhibitors. This became clear with the onset of the COVID-19 pandemic, when a large number of publications appeared on the search for inhibitors of proteins involved in the replication of the SARS-CoV-2 coronavirus that caused the pandemic. In most cases, docking was performed first, followed by experimental in vitro testing of the best candidate compounds demonstrating in docking high affinity for the target protein. Docking programs perform positioning of a ligand in the target protein and estimate the protein-ligand binding energy. The higher this energy, the more likely that a given compound will show inhibitory activity in experiments, and the more effective a drug based on such an inhibitor will be. The algorithm of most docking programs is based on the docking paradigm: the ligand binds to the target protein near the global energy minimum of the protein-ligand system. So, the problem of docking is reduced to the search for a global minimum on a multidimensional energy surface, the dimension of which is determined by the number of degrees of freedom of the system: the flexibility of the ligand and the mobility of protein atoms. When searching for inhibitors using docking, a virtual screening of databases containing many thousand or millions of ligands is performed.

Aims: There are several dozen docking programs around the world, but their accuracy is unsatisfactory. The reason for this is, firstly, that docking programs use a very simplified description of intermolecular interaction - the so-called force fields, and, secondly, in an effort to speed up docking as much as possible many simplifications are used. The use of quantum-chemical methods instead of force fields must improve docking accuracy.

Methods: We developed a supercomputer docking programs FLM performing the global optimization of the energy of protein-ligand system using the MMFF94 force field. FLM is used to implement the quasi-docking procedure in which, first, several thousand lowest energy minima are found using MMFF94, and secondly, the energies of all these minima are recalculated using the PM7 quantum-chemical program with the COSMO implicit solvent model, and as a result the global minimum of the PM7+COSMO energy is found.

Results: For a test set of protein-ligand complexes, the positions of the ligands corresponding to the global minima coincide with the positions of the ligands co-crystallized with the proteins. For these test complexes, the protein-ligand binding enthalpy is calculated using the found ligand positions, and the correlation between the calculated and measured values of the binding enthalpy is relatively high: 0.74.

Conclusions: A new supercomputer docking program is under development, in which the energy of the protein-ligand system is calculated using the PM7 method and the COSMO solvent, and a novel tensor train algorithm for global optimization is implemented.

Mathematical modeling of hemodynamics in the left atrium during atrial fibrillation

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The objective of the investigation is to predict the thrombus formation in the left atrium appendage (LAA) during the atrial fibrillation. We assume that it depends on the geometry of LAA. Providing the numerical simulations of the blood motion in the LAA we detect the stagnation zones for various shapes of the LAA using the Navier-Stokes equations which are solved with COMSOL package. However, the correlation of the stagnation zones with the thrombosis, the characteristic time of the clot formation are still open questions. The work is in progress. In the future research we intend to use the mathematical models of the blood coagulation in the case of an injury (see the pioneer works [1], [2]) and in the case of recirculated zones ([3]).

The authors are supported by the European Social Fund (project No 09.3.3-LMT-K-712-17-0003) under grant agreement with the Research Council of Lithuania (LMTLT).

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Overcoming kinase inhibitor resistance and oncogenic RAS signaling

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Major problem encountered using small molecule cancer therapeutics in clinic is that even in susceptible cancers, these drugs rarely give durable responses, almost inevitably being hampered by signaling reactivation and development of resistance. Studying the causes of this resistance has revealed severe limitations in our understanding of the network properties and molecular mechanisms that control drug responses. We show that contrary to a common opinion, feedback loops by themselves cannot restore or overshoot steady state signaling []. De novo synthesized negative feedback regulators can lead to a transient overshoot but still cannot fully restore output signaling. These findings can rationalize recent scientific and clinical disappointments that were based on the hypothesis that negative feedback loops can fully explain drug resistance. We demonstrate that there are two major means of complete, steady state revival of signaling, enabled by (1) the network topology or (2) molecular mechanisms rendering the primary drug target active again. Network topology analysis shows that at least two, activating and inhibitory, connection routes from a primary drug target to the output, must exist for complete reactivation or overshoot of steady-state output activity that existed before the inhibition [1].

Irrespective of the network topology, drug-induced overexpression of the primary drug target or druginduced increase in its dimerization or oligomerization can restore the pathway output activity. The formation of kinase homo- or heterodimers is a major course of resistance. In this constellation one protomer is drug-bound and allosterically activates the other, drug-free protomer thereby conferring resistance. The emergence of different drug affinities between protomers in a dimer has been enigmatic, but can be explained by thermodynamics [2]. A

striking example is so-called paradoxical activation of the extracellular regulated kinase (ERK) pathway by RAF inhibitors, which is caused by RAF homo- or heterodimerization. This dimerization is promoted by RAF inhibitors and amplified by mutant RAS and negative feedback regulations, but if an inhibitor does not facilitate dimerization, negative feedback can only result in a transient overshoot of the pathway activity. Exciting and counterintuitive discoveries of ways to overcome resistance were made using next generation modelling, which combines aspects of protein structure, posttranslational modifications, thermodynamics, network architecture, mutation data and dynamic reaction mechanisms [3]. As a specific example, we show that a treatment with Type I½ and Type II RAF inhibitors can counterbalance ERK pathway reactivation and concomitant drug resistance.

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