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SINGLE NEURON IMAGING AND BEHAVIOR

Pavel M. Balaban

Institute of HigherNervous Activity and Neurophysiology
Russian Academy of Sciences
Moscow, Russia

LOCAL DNA STRUCTURES AND THEIR CRUCIAL ROLE IN REGULATION OF BIOLOGICAL PROCESSES

Václav Brázda, Eva B. Jagelská, Rob Laister, Cheryl Arrowsmith

Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i.
Královopolská 135, 612 65 Brno, Czech Republic

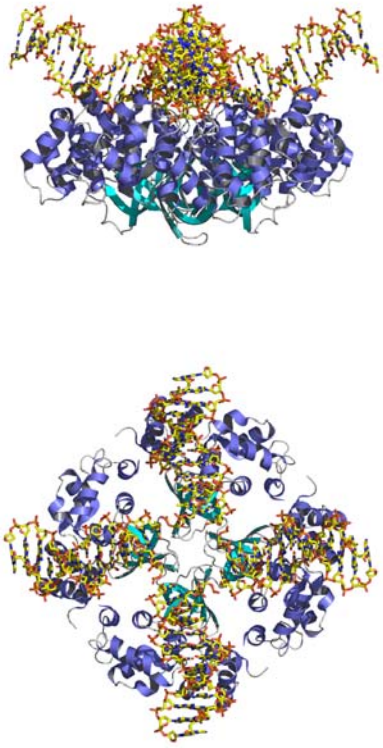
Cancer Genomics & Proteomics, Department of Medical Biophysics
Ontario Cancer Institute, Toronto
E-mail:vaclav@ibp.cz

Genome sequencing brings a huge amount of information regarding the genetic basis of life. While this information provides essentials for our understanding of biology, it has become clear that the DNA code alone does not hold all the answers. Epigenetic modifications and higher order DNA structures beyond the double helix contribute to basic biological processes and maintaining cellular stability. Local alternative DNA structures are known to exist in all organisms. Negative supercoiling induces in vitro local nucleotide sequence-dependent DNA structures such as cruciforms, left-handed DNA, triplex and quadruplex structures etc.

The formation of cruciforms requires perfect or imperfect inverted repeats of 6 or more nucleotides in the DNA sequence. Inverted repeats are distributed nonrandomly in the vicinity of breakpoint junctions, promoter regions, and at sites of replication initiation. Cruciform structures could for example affect the degree of DNA supercoiling, the positioning of nucleosomes *in vivo*, and the formation of other secondary structures of DNA. Numerous proteins have been shown to interact with cruciforms, recognizing features such as DNA crossovers, four-way junctions, and curved or bent DNA. Transient supercoils are formed in the eukaryotic genome during DNA replication and transcription, and these often involve protein binding. Indeed, active chromatin remodeling is a typical feature for many promoters and is essential for gene transcription. Several complexes that involve extensive DNA–protein interactions can only occur under conditions of negative DNA supercoiling. Interestingly, the eukaryotic genome has been shown to contain a percentage of unconstrained supercoils, part of which can be attributed to transcriptional regulation.

The three-dimensional molecular structure of DNA, specifically the shape of the backbone and grooves of genomic DNA, can be dramatically affected by nucleotide changes, which can cause differences in protein-binding affinity and phenotype. The recognition of cruciform DNA seems to be critical not only for the stability of the genome, but also for numerous, basic biological processes. As such, it is not surprising

that many proteins have been shown to exhibit cruciform structure-specific binding properties. In addition to a well-defined group of junction-resolving enzymes, we have classified cruciform binding proteins into groups involved in transcription and DNA repair (PARP, BRCA1, p53, 14-3-3, etc), chromatin-associated proteins (DEK, BRCA1, HMG protein family, topoisomerases, etc), and proteins involved in replication (MLL, WRN, 14-3-3, helicases, etc). Within these groups are proteins indispensable for cell viability, as well as tumor suppressors, proto-oncogenes and DNA remodeling proteins. Even single nucleotide polymorphisms at inverted repeats located in promoter sites can influence cruciform formation, which might be manifested through altered gene regulation. A deeper understanding of the processes related to the formation and function of alternative DNA structures will be an important component to consider in the post-genomic era.



Crystal structure of the E. coli RuvA tetramer in complex with a cruciform (PDBID 1C7Y). The Holliday junction is depressed at the center where it makes close contacts with RuvA. Each of the arms outside of the junction center takes on a standard beta-DNA conformation.

References:

Brazda V, Coufal J, Liao JC, Arrowsmith CH: **Preferential binding of IFI16 protein to cruciform structure and superhelical DNA**. *Biochem Biophys Res Commun* 2012, **422**(4):716-720.

Liao JC, Lam R, Brazda V, Duan S, Ravichandran M, Ma J, Xiao T, Tempel W, Zuo X, Wang YX *et al*: **Interferon-inducible protein 16: insight into the interaction with tumor suppressor p53**. *Structure* 2011, **19**(3):418-429.

Brazda V, Laister RC, Jagelska EB, Arrowsmith C: **Cruciform structures are a common DNA feature important for regulating biological processes**. *BMC Mol Biol* 2011, **12**:33.

Jagelska EB, Pivonkova H, Fojta M, Brazda V: **The potential of the cruciform structure formation as an important factor influencing p53 sequence-specific binding to natural DNA targets**. *Biochem Biophys Res Commun* 2010, **391**(3):1409-1414.

Brazda V, Jagelska EB, Liao JC, Arrowsmith CH: **The Central Region of BRCA1 Binds Preferentially to Supercoiled DNA**. *J Biomol Struct Dyn* 2009, **27**(1):97-104.

Jagelska EB, Brazda V, Pecinka P, Palecek E, Fojta M: **DNA topology influences p53 sequence-specific DNA binding through structural transitions within the target sites**. *Biochem J* 2008, **412**(1):57-63.

Brazda V, Jagelska EB, Fojta M, Palecek E: **Searching for target sequences by p53 protein is influenced by DNA length**. *Biochem Biophys Res Commun* 2006, **341**(2):470-477.

INFLUENCE OF MUTATIONS IN *DROSOPHILA LIMK* GENE ON CONFORMATIONAL STATE OF DNA: BRILLOUIN LIGHT SCATTERING STUDY

A.V. Dmitriev[1,2], A.I. Fedoseev[2], G.A. Zakharov[1,3]
S.G. Lushnikov[2], E.V. Savvateeva-Popova[1,3]

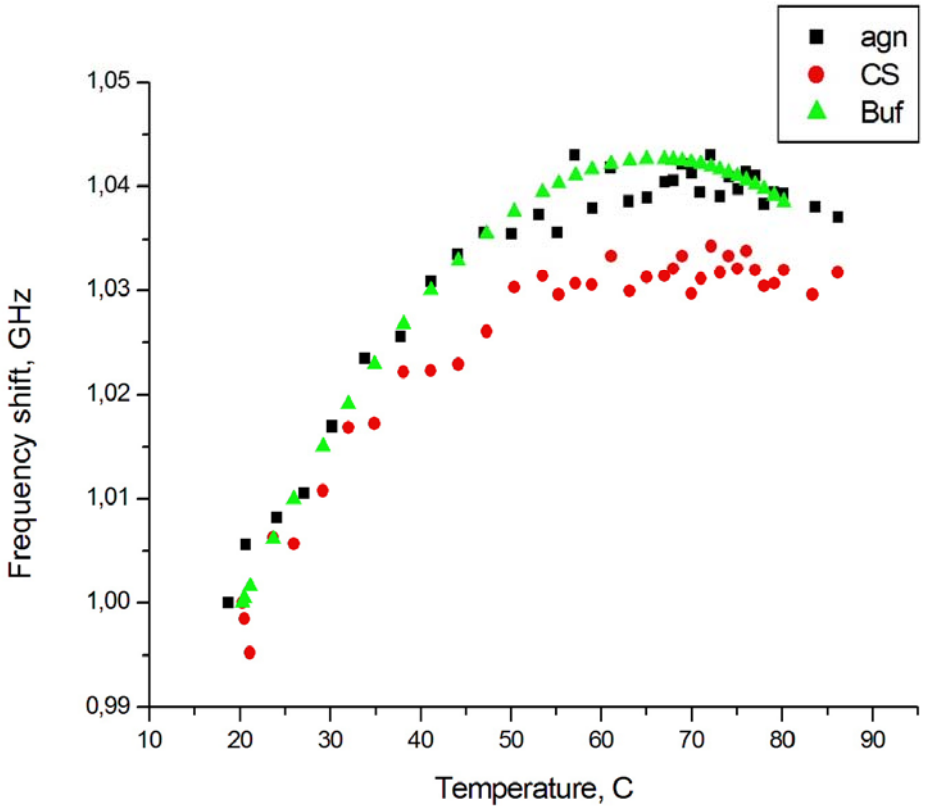
[1)] St. Petersburg State University, St.Petersburg, Russia
[2)] Ioffe Physical-Technical Institute, St.Petersburg, Russia
[3)] Pavlov Institute of Physiology, St.Petersburg, Russia
E-mail:art41090@gmail.com

During the last decade researchers actively studied phase transformations of DNA, proteins, and other biopolymers which can lead to changes in activity and functions. Phase transformations of biopolymers mean changes in the spatial structure of a macromolecule under the influence of external factors (temperature, pressure, etc.). One of the least of all studied problems is how the vibrational spectrum of a macromolecule behaves when its spatial structure changes or, in other words, what is the dynamics of a macromolecule at phase transformation. Our work was aimed to study the low-frequency (frequency range from 1 to 1000 GHz) dynamics of DNA at melting. It is known that the low-frequency dynamics of biopolymers typically reflects local conformational changes, i.e., hops of atoms from one configuration to another, as well as their collective excitations. Dynamics of biopolymers in this frequency range has many common features with the dynamics of glasses, super-cooled liquids, and other condensed media. One of the most promising techniques that give valuable information on the low-frequency dynamics of biopolymers is Brillouin light scattering.

We employed this technique to investigate the low-frequency DNA dynamics at its melting. We have got two different samples of DNA (120 bp long)with known sequence of nucleotides from the I intron of *Drosophila* LIM-kinase gene, one being from the mutant agn^{ts3} (locus *agnostic*), the other from the wild type strain Canton-S(CS). The main difference between them is the insertion of 28nucleotides in the I intron in the mutant DNA which iscapable of generating hair-pin structures to produce micro RNA partially homologous to dme-miR1006. Both DNA samples were obtained from genomic DNA by a polymerase chain reaction. The samples were a DNA solution in a sodium-phosphate buffer (pH = 7.5).

Experiments were carried out in 180^0 scattering geometry by a three-pass piezo-scanned Fabry-Perrot interferometer. The light source was an argon laser with wavelength λ = 488 nm. The objects of measurements were solutions of DNA with concentration of 400 μg/ml, the temperature range was 20–90^0C.

It has been shown that the hypersound velocity exhibits an anomaly in the region of the theoretical melting temperature. At this temperature, an anomaly in damping of hypersonic waves is also observed. Also, it has been shown that the mutant DNA is more resistant to the action of external factors, since all molecules in solution obtain the same conformation, earlier than in the wild type DNA (Fig).



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NEUTRON SCATTERING ON BIOMATERIALS – WHAT CAN WE LEARN?

Jan Peter Embs

Research with Neutrons and Muons Laboratory for Neutron Scattering
Paul Scherrer Institut; Switzerland)
E-mail:jan.embs@psi.ch

Basic neutron scattering techniques (time-of-flight spectroscopy, small-angle and quasi-elastic neutron scattering, etc.) widely used at present in the investigations of biopolymers are considered. Specific features of vibrational spectrum of biopolymers in the region of low temperatures (below -350^{o}C), where changes in the spatial organization of biopolymers, i.e., the so-called dynamic phase transition, are observed, are treated in detail. In this case properties of proteins and DNA manifest characteristic features of classical glasses. Different mechanisms of long–term relaxation, picosecond dynamics of biopolymers and a number of other manifestations of glass properties are discussed. Particular attention is given to a possibility of studying biopolymer solutions by neutron spectroscopy. This is especially important, because the majority of the data reported in literature have been obtained on powders, while neutron scattering studies of biopolymer solutions are more interesting and informative. Wide possibilities of the neutron scattering technique for analysis of different motions of atomic groups in biopolymers, biopolymer diffusion, etc., are pointed out. A necessity of a close link between mathematical modeling, neutron experiment and biological investigations is emphasized.

MOLECULAR MOBILITY WITHIN THE NEURONAL MEMBRANE

Martin Heine

Research group Molecular Physiology, Leibniz Institute for Neurobiology
Magdeburg Germany
E-mail: Martin.Heine@lin-magdeburg.de

Surface mobility within the cell membrane is involved within cell-cell recognition and adhesion. In neurons, the molecular mobility within the neuronal membrane has been described by methods as fluorescent recovery after photobleach (FRAP) or single particle tracking (SPT) and demonstrated a remarkable mobility of ionotropic and metabotropic neurotransmitter receptors and adheasion molecules within synapses. Such moleculare dynamic has consequences for short term plasticity of synapses, where the alignment of pre- and postsynaptic membranes is critical for the efficacy of synaptic transmission.

Investigating the mobility of AMPA receptors within the postsynaptic membrane and calcium channel subunits in the presynaptic membrane confirmed a very dynamic arrangement of key elements for synaptic transmission. Interference with the mobility by artificial x-link of the receptors and alpha2delta-subunits demonstrate a clear impact of this dynamic in short term plasticity. These findings suggest that the high variability of synaptic transmission between central synapses is partially caused by the fluidity of the membrane and regulate the availability and open properties of pre- and postsynaptic ion channels.

CHEMILUMINOMETRIC METHOD FOR THE ASSESSMENT OF ENVIRONMENTAL POLLUTION USING MODEL ENZYMATIC SYSTEM

Yury Kalatzky, Tatiana Petrova, Vasily Stefanov

Department of Biochemistry, St. Petersburg State University, Russia
E-mail: vastef@mail.ru

A based on the use of an enzymatic analytical test-system is proposed. The test-system responds to the presence of a variety of pollutants, regardless of their individual chemical nature and composition of mixtures. These may be organic and inorganic substances or heavy metal ions. The proposed method provides an integral quantitative estimate of the impact of aggressive environmental factors on biological objects, being easy and simple in operation, rapid, reliable and cost-effective.

The test system includes a complex multi-enzyme preparation exhibiting oxidase-peroxidase activity which functions as an open multi-target object receiving chemical signals of environmental trouble and transmitting them to the other part of the system, responsible for light generation via the induced chemiluminescence mechanism. Polluting substances, as negative signals, can realize their quenching effect using different pathways. While in the absence of polluting agents (control sample) the addition of fluorogenic substance causes light emission, pollutants, once introduced into the medium, can either directly inhibit the enzymatic activity involved in the light generation process or cause quenching of chemiluminescence interacting with the intermediate products of the enzymatic reaction. Some pollutants may act as quasi-substrates deprived of the ability to impart the capacity for generating photons to the enzyme. The way in which the quenching effect is realized depends on the specific nature of each pollutant and their composition.

Also added in the incubation medium is sulfhydryl reagent, which greatly increases the sensitivity of the system with respect to various pollutants. Sulfhydryl reagent acts in two ways: it is both a substrate for oxidation in the chain of oxidase-peroxidase reactions, and stabilizer of the multienzyme system activity. As the oxidation substrate in the oxidase-peroxidase system, sulfhydryl reagent starts a chain reaction, resulting in light emission, recorded by chemiluminometer. In the presence of sulfhydryl reagents light emission increases several orders of magnitude.

The test system is stable in a wide range of pH, temperature and concentrations of reagents in the reaction mixture. To unify the estimation pollution we introduced arbitrary units, basing on the quenching effect of induced chemiluminescence in the

polluted samples.

The main benefits of the proposed analytical system for the integral assessment of the environmental pollution:

1. Integral characterization of the environmental status (water, air, soil), which is possible due to the multiple sensitivity of the enzymatic test-system to a wide range polluting agents.
2. Quantitative characterization of the environmental status in the introduced arbitrary units of pollution.
3. Express character of the analysis suggesting fast treatment of samples for pollution assessment (4-6 min).
4. Higher (exceeding biological) sensitivity of the analytical enzymatic test-system, e.g. compared to that of the system based on bacteria.
5. Stability of the enzymatic test-system, which allows its using during long time and under different conditions, e.g. in the expedition.
6. High reproducibility of the measurements and estimates, characterized by a low value of variation coefficient (5 -8%).
7. Possibility to use modern luminometric equipment for the registration of quenching of enzymatic photochemical reaction.
8. Available complex system "measuring device – analytical kit (set of reagents) ready for use to conduct measurements both in stationary laboratory conditions and in expeditions.

The following areas for application of the developed method can be proposed:

1. Environmental mapping of different regions.
2. Location of environmentally hazardous sources.
3. Monitoring of water purifying installations.
4. Express testing of drinking water
5. Incorporation into the systems of environmental monitoring

The developed method for the integral express assessment of the environmental pollution is patented. (Patent of Russian Federation № 2359036). It was applied in the study of water pollution in the Neva River, its tributaries and small water basins in the St. Petersburg region.

pCREB AND pCOFILIN AT ADULT THORACIC NEUROMUSCULAR JUNCTIONS IN LEARNING OF *Drosophila melanogaster*

A.N. Kaminskaya[1,2,3], A.V. Medvedeva[2,3], T.L. Payalina[2,3], E.V. Savvateeva-Popova[2,3]

[1]Sechenov Institute of Evolutionary Physiology and Biochemistry of Russian Academy of Sciences, Saint-Petersburg (Laboratory of comparative physiology of sensory systems)

[2]Saint-Petersburg State University, Saint-Petersburg (Laboratory of biochemical genetics)

[3]Pavlov Institute of Physiology of Russian Academy of Sciences, Saint-Petersburg (Laboratory of neurogenetics)

E-mail: kaminskayaan@mail.ru

One of the crucial regulators of cytoskeleton remodeling is LIMK1 which phosphorylates cofilin and affects actin filament dynamics leading to dendritic spine reorganization, learning and memory. As shown previously by means of Western blot analysis, a different ratio of D and C isoforms LIMK1 in the heads of *Drosophila* males from wild type strains – *Berlin*, *Oregon-R* and *Canton-S* accompanied with similar total content of LIMK1, while mutant agn^{ts3} had high activities of LIMK1. Using setup for automatic registration of courtship song, we evaluated learning ability in two types of training condition - 30 min and 5 hours. Learning indices (LIs) was calculated based only on singing index (wing vibration produced by a male during courtship before and after learning). *Berlin* and *Oregon-R* demonstrated lower learning indices than *Canton-S* after 30 min of training, while after 5 hours - had LIs similar to *Canton-S*. agn^{ts3} had lower learning indices after both training conditions in comparison to *Canton-S*.

To estimate a possible involvement of the agn^{ts3} mutation in memory formation after 5-hr massive training we analyzed distribution pCREB and pCofilin as a markers of LIMK1 activity at adult thoracic neuromuscular junctions (NMJ) of basalar muscle involved into sound production during courtship behavior. Using confocal microscopy, we found different distribution of pCREB in *Canton-S* and agn^{ts3}. Both in *Canton-S* and agn^{ts3} pCREB was detected in thin nerve terminals but not at the synaptic boutons before learning. After learning pCREB level increased and bridges between axons were formed. In agn^{ts3} pCREB was detected in nuclei of nervous and muscular cells before and after learning. Presumably, this distinctive localization of pCREB might be promoted by alteration in levels of LIMK1 D and C isoforms in agn^{ts3}due to disturbances of nuclear-cytoplasmic transport. In addition distinctive localization of

pCREB in NMJs before and after learning can be explained by the involvement peripheral nervous system of *Drosophila* into learning process. Surprisingly, pCofilin was predominantly locolized in glial sheaves in all strains. The data are discussed in the light of new findings concerning the role of pCREB and pCofilin in NMJs as a functional node in neurobiology.

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DNA NANOSTRUCTURES AND THEIR APPLICATION IN NEW TECHNOLOGIES

Nina Kasyanenko

Faculty of Physics, St. Petersburg State University
E-mail: nkasyanenko@mail.ru

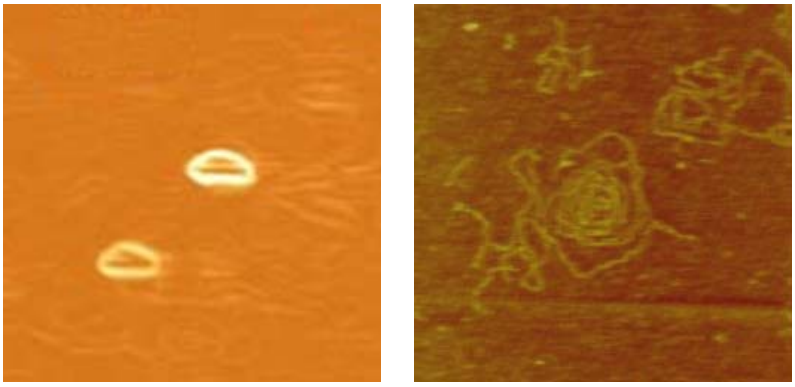
DNA molecule is a suitable material for the creation of different nanostructures due to its unique physical and chemical properties. DNA is a rigid molecule in double-stranded conformation. After melting the flexibility of DNA chain increase greatly. The complementarity of DNA bases gives the possibility to construct different structures. The negative charges of DNA phosphates lead to the polyelectrolyte swelling of molecular coil in a water solution. The screening of negative charges causes DNA condensation. DNA condensation in solution can be determined as the conformational transition of swollen molecular coil into compact state accompanied with the formation of nanodimensional particles. The study of this phenomenon is interesting not only for understanding the folding of the highly charged and rigid polymer into a compact structure, but also for DNA nanostructures design on purposes of new technologies (gene therapy, nanoelectronics, etc.)

DNA condensation induced by different agents: metal ions and polycations including poly-L-lysine, oligopeptides, polyamines (“Sigma”) and synthetic polymers which were kindly provided by Dr. O. Nazarova and Prof. E. F. Panarin (Institute of Macromolecular Compounds, St. Petersburg, Russia). Both plasmids pFL44, pRS425 in circular form and calf thymus DNA (“Sigma”) of different molecular weights were used. DNA conformation in a solution and DNA nanostructures structures were explored by the methods: Low-gradient Viscometry, Flow Birefringence, Atomic Force Microscopy, Gel Electrophoresis, Dynamic Light Scattering, UV Spectrophotometry, Circular Dichroism.

It was shown that the most important factor for DNA condensation induced by polycations (starting from 17 monomers for poly-L-lysine) is the equal concentration of the ionic groups for polycations (N) and DNA (P), i.e. N/P =1. For metal ions, oligopeptides and polyamines the realization of a definite condensing agents concentration independently on N/P is essential. On the base of experimental data phase diagrams were plotted for all systems embracing the three areas. The first area corresponds to the state of DNA coil before condensation, in the second area the formation of complexes and DNA condensation are performed, and in the third area

DNA precipitation occurs. The definite DNA conformational changes (the appearance of mutually oriented parts of DNA chain) were observed before the condensation induced by polyamines and metal ions as opposed to polymers as condensing agents.

Another nanostructures (DNA nanowires, DNA-metallic nanoparticles structures) are also discussed.



a) b)

Figure 1. AFM images of calf thymus DNA in complex with poly-L-lysine (270 monomers) (a) and $LaCl_3$ [2] (b). Scan size is 400 nm (a) and 2μm (b).

GENE EXPRESSION AND PEPTIDE THERAPY OF AGE-ASSOCIATED DISEASES

Vladimir Khavinson

Pavlov Institute of Physiology of RAS, Russia
E-mail: miayy@yandex.ru

Short peptides are a system of signal molecules regulating gene expression. Gene expression activation correlates with the specific proteins alteration.

Peptides increase lifespan of rats by about 30-40% and inhibit growth of tumors. Peptides introduced *in vivo* influence the gene expression profile. Peptides introduced to transgenic mice suppressed expression of the *HER-2/neu* mammary gland cancer gene 2-4-fold. The peptides introduced increase transcription of *IL-2* and *c-fos* genes in lymphocytes and various structures of hypothalamus. The mechanisms of the peptide action associated with chromatin activation in blood lymphocytes of aged patients. Human fibroblasts treated with peptides showed the telomerase activity induction and a 2.5-fold increase in the mean telomere length. Peptides increased the number of cell divisions by 42.5%, i.e. the overcoming Hayfiick's limit. Treatment of aged and senile people with peptides improved their physiological functions and decreased the lethality by 44-49% in a period of 8-12-year clinical observation. Peptides bind to double- and single-stranded deoxyribooligonucleotides containing CNG and CG sequences that are target sites for DNA methylation in eukaryotes. Peptides modulate specifically the action of eukaryotic endonucleases depending on DNA methylation status. The peptide modulating action on DNA hydrolysis seems to be due to site-specific peptide binding with DNA that may protect DNA against enzymatic hydrolysis. Peptide binding to CNG or CG sites in gene promoters should prevent their methylation with respective DNA-methyltransferases and leave promoters to be unmethylated that is crucial for activation of most genes. Thus, specific peptide-DNA interactions control epigenetically the cell genetic functions and can be responsible for vital resource enhancement.

LIGAND-RECEPTOR INTERACTIONS OF INSULIN SUPERFAMILY PEPTIDES

Olga Ksenofontova and Vasily Stefanov

Saint Petersburg State University
Department of Biochemistry, Russia
E-mail: jabiata@yandex.ru

Insulin superfamily is a large evolutionary-related group of vertebrate and invertebrate peptides, which consists of insulin, insulin-like growth factors (IGF1 and IGF2), bombyxin, relaxin, and insulin-like peptides[1]. Insulin is well known for its role in the regulation of metabolic activity: it induces the conversation of glucose to glycogen, inhibits gluconeogenesis, and shows an indirect anabolic effect on lipid and protein metabolism[2]. It is known that the primary and tertiary structures are very similar among insulin superfamily peptides. This property is actively used for developing new pharmacologically important insulin analogues, which has some amino acid substitutions in the primary structure. For example, insulin lispro has reverse penultimate lysine and proline residues at positions B28 and B29 (like in IGF1)[3]. This modification does not alter insulin-receptor binding, but inhibit the formation of insulin dimers and hexamers.

Using direct drug delivery systems, such as polymer or nanoporous capsules[4], it is not necessary to inhibit hexamers formation. But it is very important to enhance insulin-receptor binding. The aim of our work was to investigate ligand-receptor interactions and stability of ligand-receptor complexes, formed by insulin receptor and modified insulin molecule. We used ThrA8 as a target of amino acid substitutions due to its natural variability among peptides of insulin superfamily[5]. We chose His, Lys, Glu, and Phe. Thus, in our computational experiments we changed polarity and charge. All substitutions, except Phe, have natural occurrence among vertebrate insulin sequences. PheA8 is typical for IGF1, IGF2, and some insulin-like peptides.

In our work we used two computational methods – molecular dynamics (MD) simulation and docking. First, we carried out MD for molecular relaxation, using GROMACS software package. After that we performed ligand-receptor docking between insulin receptor and modified insulins. The starting structure of the human insulin was obtained in Protein Data Bank (PDB code 1BEN). The homology models of modified insulins have been constructed using the MODELLER software. Obtained with the MODELLER molecules, we immersed in solvent using tip4p water model. The solvated proteins were placed in the cubic boxes with periodic conditions and the

system's energy was minimized to remove steric clashes. To compute the forces acting on each atom in reasonable, OPLS-AA force field was applied.

Comparison of the different physical and chemical parameters, obtained in docking, showed that His and Lys substitutions were the most effective for the insulin receptor binding. Furthermore, this substitutions contributed in ligand-receptor complex stability in MD carried out after docking. These results are in a good agreement with the experimental data. Glu substitution was less effective for binding. Probably, it can be explained by existence of a negative charge at A8 position. Interestingly, all modified insulins showed native-like tertiary structure and no changes observed in disulfide binding nearby cysteines. Because His and Lys have greater intrinsic alpha-helical propensity than Thr and exhibit enhanced affinities and stabilities we proposed that this peptides are the best candidates for the oral clinical application in the polymer or nanoporous capsule.

References:

1. Q. Hua, J.P. Mayer, W. Jia, J. Zhang, M.A. Weiss. *The folding nucleus of the insulin superfamily. A flexible peptide model foreshadows the native state*, The Journal of Biological Chemistry. V. 281. P. 28131-28142, 2006.

2. I. Claeys, G. Simonet, J. Poels, T.V. Loy, L. Vercammen, A.D. Loof, J.V. Broek. *Insulin-related peptides and their conserved signal transduction pathway*, Peptides. V.23. P. 807-816, 2002.

3. C. Mclnnes and B.D. Sykes. *Growth factor receptors: structure, mechanism, and drug discovery,* Peptide Science. V. 43. P. 339-366, 1998.

4. M. Arruebo. *Drug delivery from structured porous inorganic materials*, Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. V. 4. P. 16-30, 2012.

5. Y. Yang, A. Petkova, K. Huang, B. Xu, Q. Hua, I. Ye, Y. Chu, S. Hu, N. Phillips, J. Whittaker, F. Ismail-Beigi, R.B. Mackin, P.G. Katsoyannis, R. Tycko, M.A. Weiss. *An Achilles' heel in an amyloidogenic protein and its repair. Insulin fibrillation and therapeutic design*, The Journal of Biological Chemistry. V. 285. P. 10806-10821, 2010.

MECHANISMS OF THE PEPTIDERGIC REGULATION OF IMMUNOGENESIS: MOLECULAR MODELING *in silico*

Natalia Linkova, Alexandr Dudkov

Saint-Petersburg Institute of Bioregulation and Gerontology, Russia
E-mail: miayy@yandex.ru

Age-related changes in the thymus play a key role in weakening of the immune system. Molecular mechanisms of thymus involution during its aging process are associated with the violation of differentiation, proliferation and apoptosis of T-lymphocytes. Dipeptide vilon (Lys-Glu), which was synthesized in the St. Petersburg Institute of Bioregulation and Gerontology, helps to recover immunogenesis in the thymus, which is reflected in its immunomodulatory, anti-allergic and oncostatic action. It was established, that vilon restores the structural organization of the thymus layers in accelerated aging, stimulates the maturation of T-cells in thymusless animals.

It was shown in dissociated and organotypic cultures of thymus cells of humans and animals that the molecular mechanism of biological activity of vilon is associated with its ability to stimulate the expression of signaling molecules – markers of differentiation (CD4, CD5) and proliferation (Ki67), and inhibit the expression of apoptotic marker (p53). In addition, it was revealed by microarray technology that vilon is able to regulate the level of expression of several genes. On the basis of experimental data a model of interaction with the promoter vilon zones of genes that regulate the maturation of T-cells was produced.

We used the method of molecular mechanics. For preliminary selection of the main and the most energetically favorable conformations of the peptide Lys-Glu we used procedures of molecular dynamics, reproducing motion of isolated molecules in given time interval. The main results of molecular dynamics were obtained in the force field MM+. The energy of minimizing here means the energy-optimal state of the molecule. In each mode we analyzed about 100 preliminary conformations of the peptides, and then out of them we selected the energy-optimal rotamers.

The sequence of the gene 5'-d(GCAG)-3'•5'-d(CTGC)-3' was modeled using DNA structure from the PDB database 1QMS. During calculation of the energy minimization of the DNA-peptide complex the influence of the solvent was considered in the generalized Born approximation with the introduction of the internal dielectric constant equal to 1 and the outer constant equal to 80. Calculations of the complexes were conducted in the AMBER99 force field. The energy of peptide binding to the DNA was

calculated as the difference between the energies of individual molecules of the DNA and the peptide and the DNA-peptide complex. The peptide Lys-Glu may receive the total charge of -1. The hydrophobicity index for the peptide was -7.4. On the molecular surface of the double helix the major and the minor grooves, having width of 2.1 nm and 1.2 nm correspondingly, were distinct. It turned out that the peptide Lys-Glu interact with the DNA by binding to its major groove. During interaction of Lys-Glu with GCAG sequence and complementary to it CGTC we have found three hydrogen bonds: the amino group of the main chain of lysine forms hydrogen bonds with the oxygen atom of guanine and nitrogen atom of cytosine, while the main chain carboxyl group of glutamic acid forms a hydrogen bond with the nitrogen atom of adenine. The energy of the biding of this peptide was 11 kcal/mol. The sequence GCAG, to which according to the modeling peptide Lys-Glu binds, was found in the promoter region of genes, modulating the transmembrane proteins CD4, CD5, Ki67, p53, being a marker of the immune cells station.

The structural models, obtained by the molecular modeling, and revealed peculiarities of the interaction of peptide Lys-Glu with nitrogen bases of GCAG sequences confirm previous suggestions about the possibility and nature of the formation of complexes of peptides and nucleic acids.

CRITICAL BEHAVIOR OF PROTEINS AT PHASE TRANSFORMATIONS

Sergey G. Lushnikov, Vadim P. Romanov

Ioffe Physical Technical Institute, RAS

St. Petersburg State University; Russia

E-mail: sergey.lushnikov@mail.ioffe.ru

The thermal denaturation of proteins (lysozyme, BSA) has been studied using Brillouin light scattering. An anomalous temperature behavior of the velocity and damping of hypersound, which is accompanied by a decrease in the intensity of the Brillouin components in the experiments with the 180° scattering geometry and almost complete their disappearance in the case of the 90° scattering geometry for lysozyme, has been observed at the sol–gel transition in the vicinity of 343 K. Such anomalies in the light scattering spectra are absent from a sodium acetate buffer used to prepare protein solutions. A mechanism describing the critical behavior of the intensities of the Brillouin components has been proposed.

INTER-ATOMIC DISTANCES IN PROTEIN STRUCTURES: STERIC REPULSION APPROXIMATIONS BASED ON REAL PROTEIN STATISTICS

Grigorii R. Mavropulo-Stoliarenko, Vasily Stefanov

Biochemistry Department, Saint-Petersburg State University
St.Petersburg, Russia
E-mail: gm2124@mail.ru

Motivation

Exact knowledge of atomic van der Waals radii in lengthy biological macromolecules, like proteins, has already been for some time (Richards, 1974; Chothia, 1975) and still is (Seeliger and de Groot, 2007) an important and somewhat debated issue in biochemistry and bioinformatics. Main reason why this topic gets repeatedly brought out to daylight is that this information is directly applicable in many areas of protein structure modeling, like:

- packing density and solvent accessible surface area calculations;
- steric constraints checking for known structures;
- random or some “potential function” guided protein structure generation.

Almost 100 years apart from the time point when first bits of crystallographic X-ray diffraction data were captured on film, lots of research on the problem has been reported: starting from classical work by Bondi (Bondi, 1964) with calculations based on first “most reliable X-Ray diffraction data” of simple molecules, and up to most recent communications by Tsai (Tsai *et al.*,1999) and Seeliger (Seeliger and de Groot, 2007), who performed their analysis on “small molecule organic structures from the Cambridge Structural Database” and fine-resolution subset of Protein Data Bank representative set correspondingly.

Main drawback of all previous research in the field is that they were focused on the idea of acquiring some set of idealized "hard sphere" approximations of the atom radii, thus leaving out questions of allowable sphere intersection and probabilistic magnitude of such violations in real protein structures. While being principally correct for the tasks of volume and surface area calculations, the idea of a point estimation of atomic radiuses is the kind of model simplification that makes it unusable for the task of random structure generation. As the consequence of this lack of information authors of the research papers that make use of atomic radii to estimate “random coil” protein state use different cutoff values for disallowed amount of atom sphere intersections, ranging from 95% (Pappu *et al.*, 2000) to 75% and below (Feldman and Hogue, 2000; Cheng *et*

al., 2007), and such decisions are usually postulated without any justification for their choice.

So, *the aim* of our research was to provide a justified probabilistic description of close atom surroundings, in terms of minimal inter-atomic distances and probability-distance distributions of the contacting atoms.

Method

To increase sample size for our study we have decided not to filter out whole structures based on the resolution factor, but instead to filter out single residues which contain covalent bonding errors, thus using for inter-atomic distance analysis only sequential error-free segments. The rationale for this choice was in preliminary observation that such error-free segments almost didn't contain heavy atom clashes.

Second important feature of our method is "zero assumption" imperative, meaning that prior to any analysis we didn't make any numeric assumptions neither about atom radii, nor about extent to which they can be violated. Also we didn't place strict arbitrary restrictions on maximal distance cutoff of analyzed atom pairs, only limiting (for performance reasons) our analysis to 100 closest partners for each atom. Filtering of the "non contacting" atoms was performed in an iterative procedure based on some simple geometric considerations and minimally allowed contact distances that were acquired during earlier iterations. This filtering process is somewhat similar to the process of constructing Voronoi polyhedra during atomic volume calculations.

Finally, since hydrogen atoms are not usually visible on the electron density map and since that it is widely adopted by the community that hydrogen atoms can be seen as partially "submersed" into the outer electron orbitals of covalently bound heavy atoms, here we were using atomic group representations of heavy atoms instead of treating each hydrogen separately.

Results

We present a set of minimal inter-atomic distances, derived from analysis of more than 4k protein chains comprising ~600 000 amino acid residues, taken from the X-RAY part of PDBselect 25 representative Protein Data Bank reference set (Hobohm *et al.*, 1992). These minimal distances can be viewed as the sum of "hard sphere" radii of corresponding atoms, in the strict understanding of the "hard sphere" term. Also we provide probabilistic descriptor for each item in a minimal distance set. That descriptor can be used as a soft cutoff atomic repulsion term in random structure generation.

We also show that our set of inter-atomic distance descriptors can give a unified view at the known diverse point estimations of der Waals radii.

References:

1. Bondi A. van der Waals Volumes and Radii. J. Phys. Chem., 1964, 68 (3), pp 441–451
2. Cheng J, Pei J, Lai L. A free-rotating and self-avoiding chain model for deriving statistical potentials based on protein structures. Biophys J. 2007 Jun 1;92(11):3868-77. Epub 2007 Mar 9.
3. Chothia C. Structural invariants in protein folding. Nature. 1975 Mar 27;254(5498):304-8
4. Feldman HJ, Hogue CW. A fast method to sample real protein conformational space. Proteins. 2000 May 1;39(2):112-31.
5. Hobohm U, Scharf M, Schneider R, Sander C. Selection of representative protein data sets. Protein Sci. 1992 Mar;1(3):409-17.
6. Pappu RV, Srinivasan R, Rose GD. The Flory isolated-pair hypothesis is not valid for polypeptide chains: implications for protein folding. Proc Natl Acad Sci U S A. 2000 Nov 7;97(23):12565-70.
7. Richards FM. The interpretation of protein structures: total volume, group volume distributions and packing density. J. Mol. Biol. 1974;82:1-14.
8. Seeliger D, de Groot BL. Atomic contacts in protein structures. A detailed analysis of atomic radii, packing, and overlaps. Proteins. 2007 Aug 15;68(3):595-601.
9. Tsai J, Taylor R, Chothia C, Gerstein M. The packing density in proteins: standard radii and volumes. J Mol Biol. 1999 Jul 2;290(1):253-66.

FUNCTIONAL MAGNETIC RESONANCE IMAGING AND NEUROSCIENCES

Ksenia Mazhirina[1,3], Michail Pokrovsky[3], Maria Rezakova[2], Andrey Savelov[2], Mark Shtark[1,3]

[1] Institute of molecular Biology and Biophysics SB RAMS, Novosibirsk

[2] International Tomography Center SB RAS, Novosibirsk

[3] Computer systems of biocontrol, Novosibirsk

E-mail: as@tomo.nsc.ru

Functional magnetic resonance imaging (fMRI) is a group of methods within a class of intrascopic technologies using magnetic phenomena. It focuses primarily not on the anatomical details of brain structures, but provides an alternative view of involvement of different cortex areas in specific tasks. Based on Blood Oxygenation Level Dependent (BOLD) contrast, fMRI is able to present real-time maps of oxygen supply and consumption, which are interpreted as activation/deactivation of the corresponding areas.

Main advantages of fMRI are high spatial resolution and noninvasivness, which allows multiple dynamic study of the same subject. Since its invention in 1990 [1] fMRI has been successfully used to assess a broad range of human abilities, from basic finger tapping to rather sophisticated experiments which reveal e.g. temporal effectiveness of short-term memory. Very fine details of human mental processes may be directly detected and quantitatively described in terms of statistical parameter maps. Relatively high spatial resolution of fMRI images provides a valuable guide for neurosurgery, especially when combined with fiber tracking technologies. Efficiency of blood supply is immediately visible on fMRI, allowing early detection of stroke risks. Psychologists may study such things as ability to learn, mental disorders like autism and behavioral deviations. Complex interactions between psychological and physiological events may be investigated, with few known alternative methods.

Using fMRI we expect to gain insight on the process of neuronal ensembles formation in Brain-Computer-Interface paradigm. We investigate neuronal responses formed during biofeedback-driven computer race games, where win/loss chances depend on physiological parameters, like heart rate. It may be shown that motivated humans are able to quickly learn control over such parameters, consciously invoking activations of brain areas responsible for corresponding activity. Typically, after a few trial-and-fail attempts one finds a right way to win the race. FMRI captures this

dynamic showing mass activation of cortex areas in early stages, followed by formation of specialized network or neuronal ensemble. Once found, this network persists and may be reused for a prolonged period of time.

Another field of our interest is the quantification of hemodynamic response function. There is a well-known “balloon” model of blood supply during brain activation [2]. This model includes a number of biomechanical characteristics of vessel pool elasticity, oxygen content and extraction rate, combined into a set of differential equations. We believe it possible to fit experimentally measured BOLD response to this model and obtain physiologically meaningful data related to the efficiency of regional blood supply. Quantitative characteristics of the brain tissue state may be used as indicators of ischemia risk, specific diseases including Alzheimer and Parkinson's, for assessment of results of drug treatment etc.

fMRI is a proven tool for efficient investigation of brain activity and pathological conditions, but its development is far from being complete. We expect further growth of interest to this unique method among health care specialists.

References:

[1] Ogawa S et al, Oxygenation-sensitive contrast in magnetic resonance imaging of rodent brain at high magnetic fields // Magn. Reson. Med. 1990. V14(1). P.68-78.

[2] Friston KJ et al, Nonlinear responses in fMRI: Balloon model, Volterra kernels and other hemodynamics. // NeuroImage 12. 2000. P.466-477.

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A TOOL FOR *IN VIVO* ASTROCYTIC ACTIN MANIPULATION UNDER TWO-PHOTON MICROSCOPE

Dmitry Molotkov, Svetlana Zobova and Leonard Khiroug

Neuroscience Center University of Helsinki, Finland
E-mail: dmitry.molotkov@helsinki.fi

Peripheral astrocytic processes (PAPs) are highly motile component of a tripartite synapse (Perea et al., 2009; Reichenbach et al., 2010). PAPs are also known to contain actin-related protein Ezrin (Lavialle et al., 2011) suggesting actin-based motility mechanisms. However, the link between dynamic activity-dependent changes in astrocytic morphology and synaptic function has not been established experimentally due to lack of selective tools. We developed a bicistronic cassette for decelerating the motility of PAPs and simultaneous morphology tracing. It facilitates overexpression of actin binding protein Profilin-1 with single amino acid substitution that prevents its binding to actin monomers (Suetsugu et al., 1999) and of a membrane-targeted Lck-GFP (Benediktsson et al., 2005). Astrocytes expressing the cassette showed a significantly reduced PAPs outgrowth rate in a response to stimulation by Ca^{2+} uncaging. On the other hand basal motility rate, static morphology and major cytoskeletal structures were unchanged in astrocytes overexpressing mutant Profilin-1 compare to control. The mutant Profilin-1/Lck-GFP expressing cassette virally delivered to the mouse brain is a new tool for studying synapse-PAPs interplay using *in vivo* two-photon microscopy.

HETEROLOGOUS PROTEIN PRODUCTION IN YEAST *Pichia pastoris*

M.V. Padkina

Laboratory of Biochemical Genetics, Department of Genetics and Biotechnology
Saint-Petersburg State University, Russia
E-mail: mpadkina@mail.ru

Nowadays natural proteins with highly desirable biological properties can be produced efficiently in microorganisms. It is worthy of note that bacteria lack the ability to perform many eukaryote-specific post-translational modifications and often produce eukaryotic proteins that are misfolded, insoluble or inactive. Furthermore, the bacterial derived recombinant proteins can contain the contaminants (particularly endotoxins). The advantages of yeast as a host for the expression of recombinant proteins from higher eukaryotes have long been appreciated. They combine the ease, simplicity and cheapness of bacterial expression systems with low level of contaminants and the authenticity of the far more expensive and less convenient animal tissue culture systems. Methylotrophic yeast *P. pastoris* has rapidly become the system of choice for the production of the proteins of human, animal and plant origin. *P. pastoris* has the potential for growth to very high cell densities, high expression levels and efficient secretion increase essentially the yield of recombinant protein in comparison with yeast *S. cerevisiae*. It is known, that proteins secreted by *P. pastoris* are not hyperglycosylated as is often the case in *S. cerevisiae* and do not contain α1-3-bonded mannose residues which are the strong antigenic determinants. Hence *P. pastoris* can produce authentic extracellular proteins in parallel with the intracellular ones. On this basis the recombinant proteins produced by *P. pastoris* and secreted into growth medium can be used as therapeutics.

We use yeast expression system to produce some therapeutic proteins such as human and animal cytokines. Cytokines are responsible for broad spectrum of biological activities in organism. They prime the host immune response and provide an effective antineoplastic- and antiviral-immune response. Cytokines, as natural mediators and regulators of the immune response, offer exciting alternatives to the conventional chemical-based therapeutics.

By now we originally have constructed the yeast *P. pastoris* strains capable of high level synthesis and secretion of the recombinant human α16-interferon, β-interferon, γ-interferon, interleukin-2, bovine and chicken γ-interferons. In addition we originally have constructed the yeast *P. pastoris* strains secreting recombinant proteins

derived from gene fusion of human serum albumin and protein of interest (human α16-interferon, β-interferon, interleukin-2). It is known that conjugation to serum albumin have been used to improve the pharmacological properties and to expand the in vivo half-life of recombinant proteins. There were the positive results of pre-clinical trials of human α16-interferon, β-interferon. It was shown that recombinant interferons do not make toxic action on animal organism. No allergic features have been found. It was revealed pronounced antiviral effect of recombinant interferons.

DEVELOPMENT-DEPENDENT REGULATION OF SYNAPTOGENESIS AND SYNAPTIC PLASTICITY VIA SEROTONIN RECEPTORS

Evgeni Ponimaskin

Department Cellular Neurophysiology
Medical School Hannover, Germany
E-mail: Ponimaskin.Evgeni@mh-hannover.de

The common neurotransmitter serotonin controls different aspects of early neuronal differentiation, although the underlying mechanisms are poorly understood. Here we report that activation of the serotonin 5-HT7 receptor promotes synaptogenesis and enhances synaptic activity in hippocampal neurons at early postnatal stages. An analysis of Gα12-deficient mice reveals a critical role of G12 protein for 5-HT7 receptor-mediated effects in neurons. In organotypic preparations from the hippocampus of juvenile mice stimulation of 5-HT7R/G12 signaling potentiates formation of dendritic spines, increases neuronal excitability and modulates synaptic plasticity. In contrast, in older neuronal preparations morphogenetic and synaptogenic effects of 5-HT7/G12 signaling are abolished. Moreover, inhibition of 5-HT7 receptor had no effect on synaptic plasticity in hippocampus of adult animals. Expression analysis reveals that the production of 5-HT7 and Gα12 proteins in the hippocampus undergoes strong regulation with a pronounced transient increase during early postnatal stages. Thus, regulated expression of 5-HT7 receptor and Gα12 protein may represent a molecular mechanism by which serotonin specifically modulates formation of initial neuronal networks during early postnatal development.

MECHANISMS OF THE PEPTIDERGIC REGULATION OF CELL DIFFERENTIATION IN THE RETINA: MOLECULAR MODELING *in silico*

Valeriya Pronyaeva, Svetlana Trofimova

Saint-Petersburg Institute of Bioregulation and Gerontology, Russia
E-mail: lerapronyaeva@gmail.com

Age-related retinal degenerative diseases are the cause of reduced vision and blindness. It was established, that geroprotective peptide epitalon (Ala-Glu-Asp-Gly), which was synthesized in the St. Petersburg Institute of Bioregulation and Gerontology, increases the life span, memory and restores visual function to people over 60 years. The aim of the research was to study the effect of epitalon on the expression of markers of differentiation for determination of peptidergic regulation.

It was shown in organotypic cultures of retinal cells of chick embryo that the molecular mechanism of biological activity of epitalon is related with its ability to stimulate the expression of signaling molecules – markers of differentiation retinal cells (Vsx1, Chx10, Pax6, Brn3, Math1, Prox1, TTR). On the basis of experimental data a model of interaction with the promoter epitalon zones of genes that regulate the maturation of retinal cells was produced.

We used the method of molecular mechanics. For preliminary selection of the main and the most energetically favorable conformations of the peptide Ala-Glu-Asp-Gly we used procedures of molecular dynamics, reproducing motion of isolated molecules in given time interval. The main results of molecular dynamics were obtained in the force field MM+. The energy of minimizing here means the energy-optimal state of the molecule. In each mode we analyzed about 100 preliminary conformations of the peptides, and then out of them we selected the energy-optimal rotamers.

The sequence of the gene 5'-d(ATTTC)-3'•5'-d(GAAAT)-3' was modeled using the DNA structure from the PDB database 2EZD. During calculation of the energy minimization of the DNA-peptide complex the influence of the solvent was considered in the generalized Born approximation with the introduction of the internal dielectric constant equal to 1 and the outer constant equal to 80. Calculations of the complexes were conducted in the AMBER99 force field. The energy of peptide binding to the DNA (E, kcal/mol) was calculated as the difference between the energies of individual molecules of the DNA and the peptide and the DNA-peptide complex. In terms of physical and chemical properties, performed assessment of the extent of protonation show that the peptide Ala-Glu-Asp-Gly may receive the total charge of -2. The

hydrophobicity index was calculated for Ala-Glu-Asp-Gly -5.6. On the molecular surface of the double helix the major and the minor grooves, having width of 2.1 nm and 1.2 nm correspondingly, were distinct. It turned out that the peptide Ala-Glu-Asp-Gly interacts with the DNA by binding to its major groove. It is evident that the interaction of the peptide Ala-Glu-Asp-Gly with 5'-d(ATTTC)-3' sequence and complementary to it 5'-d(GAAAT)-3' was carried out by van der Waals, electrostatic interactions and hydrogen bonds between functional groups of both molecules. The energy of the complex formation was 10.3 kcal/mol. ATTTC sequence, which is complementary to peptide Ala-Glu-Asp-Gly, was found in the promoter regions of Vsx1, Chx10, Pax6, Brn3, Math1, Prox1, TTR genes.

The structural models, obtained by the molecular modeling, and revealed peculiarities of the interaction of peptide Ala-Glu-Asp-Gly with nitrogen bases of 5'-d(ATTTC)-3'•5'-d(GAAAT)-3' sequence confirms previous suggestions about the possibility and nature of the formation of complexes of peptides and nucleic acids.

THEORETICAL MODELING OF PROTEIN-PROTEIN AND LIGAND-PROTEIN INTERACTIONS *in silico*. MOLECULAR MECHANISM FOR PROTEIN KINASE A Iα A-DOMAIN ACTIVATION

Olga N. Rogacheva, Vasilii E. Stefanov, Boris F. Shchegolev, Elena V. Savvateeve-Popova

St. Petersburg State University

Pavlov Institute of Physiology, RAS

St. Petersburg, Russia

E-mail: acerlaetum@yandex.ru

Protein-protein and ligand-protein interactions can be studied by wide spectrum of in silico methods from bioinformatics to QM/MM (Quantum Mechanics/Molecular Mechanics). We use some of these methods, such as protein-ligand docking (Quantum 3.3.0 software) and molecular dynamics (NAMD program), to analyze formation of ligand-protein complex between cAMP (3',5'-cyclic adenosine monophosphate) and Protein Kinase A Iα A-domain and to determine the crucial stages of subsequent conformational transition of this complex.

PKA (Protein Kinase A) Iα is Ser/Thr kinase, which works as effector for cAMP increase. In its inactive state, PKA Iα exists as two dimers each comprising a regulatory (R) subunit and a catalytic (C) one. R-subunit consists of two homological cAMP-binding domains A and B, which both make contacts with C-subunit. cAMP binding via causing conformational transition of discrete A- and B-domains leads to subunits dissociation and, as a result, activation of kinase. This process is of great importance for cell functioning and drug design. However the mechanism underlying PKA domains conformational changes remains unclear in spite of intensive study over the last few decades. We tried to clarify this mechanism using the abovementioned group of computational methods.

Our data suggest that conformational transition of A-domain from C-subunit-bound form (H-form) to cAMP-bound form (B-form) proceeds preferably in a single way. The first stage of this way comprises formation of a hydrogen bond between cAMP and A202 and subsequent reorganization of phosphate binding cassette (domain structure which carries A202 and binds cAMP) into B-form. The second stage proceeds in accordance with hinge mechanism suggested by H. Rehmann (Rehmann H. et al. (2003) *Nature Structural Biology*, **10** (1), 26- 32). During the third stage one turn of the B/C-helix undergoes transformation from α- to π-helix. And the last stage consists in

substitution of this π-helical turn by kink, which separates B- and C-helices. All this stages are accompanied by N3A-motif displacement.

It is important that a small percentage of analyzed conformational transitions proceed in the way characterized by reverse order of the second and the third stages. Some mutations, such as L233A, make this mechanism preferable.

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FLUORESCENT STUDIES OF FULLERENE C60 COMPOUNDS IN BIOMOLECULAR SYSTEMS

R.V. Romanov[1], K.S. Semenov[2], N.A. Charykov[2], V.E. Stefanov[1]

[1] St.Petersburg State University, Biology-Soil Faculty

[2] ILIP Ltd, 197022 St.-Petersburg, Russia

E-mail: rrouman@mail.ru

The unique chemical nature of fullerenes accounts for considerable interest in the study of their effects on the biological substrates. In this connection the development of procedures for fullerenes solubilization and the preparation of their water soluble derivatives are of special significance. There is some evidence that fullerenes can be transferred to the water phase by incorporating them into micelles and liposomes or by preparating of the water soluble derivatives. This has made it possible to study the action of fullerenes on the respiratory activity of the liver cells and on hydrophobicity of the microsomal membranes of hepatocytos.

The effects of C60 water soluble derivatives (C60-WD) on the structure and the permeability of the phosphatidylcholine liposomes were studied both from the changes of the pyrene fluorescence spectra and using the kinetic measurements of phosphorescence quenching of the erythrosine triplet probes. It is known that pyrene introduced into liposomes localizes inside the lipid bilayer. In this case the fluorescence spectrum of pyrene contains two typical bands at 390 and 460 nm, due to monomer and excimer states of the pyrene molecules respectively. The addition of the 10^{-5} M C60-WD water solution to the suspension of liposomes leads to the quenching of the pyrene fluorescence. In this case the same decrease of the intensities of the monomer and excimer bands is observed in the spectrum, i.e., their intensities ratio does not change. This demonstrates the ability of C60-WD to penetrate into the pyrene localization region without essentially changing the structure of the lipid bilayer.

A method of the triplet probe has been elaborated to study permeability of lipid bilayer of phosphatidylcholine liposomes to chemical compounds and the effect of these compounds on the liposome integrity. The greater life time of the excited triplet states of the probes in the solutions (~10^{-4} s) as compared with the fluorescence quenching times (~10^{-8} s) provides a much higher sensitivity of the measurement of the diffusive collision between triplet probe molecules and those of the quenchers. Since the quenching of the erytrosine phosphorescence at the inner membrane side can be realized only at the probe-quencher diffusional contact, this clearly demonstrates that quencher's

molecules penetrate through the lipid bilayer. In the case of the amino acid derivatives of fullerene C60 this approach provides the opportunities for an investigation of the permeability of the lipid bilayer for the compounds under consideration, if they are quenchers of the triplet probes. When the compound itself is not phosphorescence quencher, one can observe its effect on the membrane by preintroducing into the external liposome volume an efficient phosphorescence quencher (for example Co2+-ions), incapable of penetrating through the membrane on its own.

In our study of the effect of C60-WD on liposomes it has been demonstrated that C60-WD are efficient quenchers of the excited triplet state of erythrosine in liposomes. On subsequent titration of the liposomes suspension by C60-WD solutions the intensity of the erythrosine phosphorescence decreases. This fact attests to substantial permeability of the lipid bilayer to C60-WD without violating the liposome integrity.

PROBLEMS OF QUANTITATIVE FLUORESCENT IMAGING OF Ca^{2+} SIGNALING IN BRAIN SLICES SURVIVING UNDER MICROSCOPE

D.G. Semenov, A.V. Belyakov

Pavlov Institute of Physiology, Russian Academy of Sciences
Lab. of Regulation of Brain Neuron Functions
(Head Prof. M.O. Samoilov)
Saint Petersburg, Russia
E-mail: dsem50@rambler.ru

Molecular mechanisms forming posthypoxic states of mammalian brain are the main subject of research of our laboratory. Transient disturbance of oxygen and/or glucose supply of brain neurons can induce either cell damage and death or cell survival, often followed by the increase of their resistance to the subsequent and stronger insults. The choice between these two options depends on the shape, strength, duration, location etc. of the hypoxic (ischemic) insult. The latter phenomenon is known as hypoxic preconditioning while its consequence is known as induced hypoxic tolerance. The certain dynamics of intracellular Ca^{2+} content mostly determined by activity of glutamate receptors are acknowledged as a key factor switching posthypoxic events between these alternative outcomes. The changeable level of intracellular Ca^{2+} concentration, the amplitude and frequency of its oscillation, pathways and addresses of Ca^{2+} transients and distribution of Ca^{2+} between ionized and functionally bound pools are indices underlying the Ca^{2+} dynamics.

The quantitative imaging of Ca^{2+}-associated fluorescence seems to be the most popular modern technique for measurement of intracellular Ca^{2+} dynamics in the living brain tissues. Confocal micropfluorimetry being used for recording of Ca^{2+} signals in simple and quite artificial models of nervous system has reached good results. Unfortunately it demonstrates rather modest results when incubating mammalian brain slices, which are the most useful subject for investigation of induced hypoxic tolerance, are under study.

Our experience of studying Ca^{2+} signals in perfused cortical and hippocampal slices under confocal microscope reveals a number of difficulties (stabilization of hydro-mechanical parameters of perfusion, calculation of the applied drugs concentration, choice of pares of fluorescent dyes excited by two simultaneously firing lasers, choice of settings for the most effective detection of the quick Ca^{2+} transients etc). Finally the very inverting principle typical for confocal microscopes demonstrates considerable defects when studying perfused living tissues. Some of the above mentioned defects can

be avoided by using noninverted contact microscopy with the opack-illumination. However this technical approach to the quantitative fluorescent imaging, also used in our laboratory, requires additional equipment and computer automatization. The results obtained by these two methods, the field of technical tasks and the search for their solution are under discussion.

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SHEDDING LIGHT ON THE ASSEMBLY OF SYNAPSE STRUCTURE AND FUNCTION

Stephan Sigrist

Institute for Biology/Genetics, Free University Berlin; Germany
E-mail: stephan.sigrist@fu-berlin.de

The majority of rapid cell-to-cell communication mechanisms and information processing within the nervous system makes use of chemical synapses. Thereby, the molecular organization of presynaptic active zones, the places where neurotransmitter filled synaptic vesicles get released, is a focus of intense investigation. We recently showed that Bruchpilot, which features homologies to the mammalian CAST/ERC family, is essential for structural organization and efficient neurotransmitter release at active zones (*1, 2*).

Our group established protocols to directly visualize protein dynamics during synapse assembly and plasticity in living intact larvae over extended periods (*3-5*). Here, we adapted a recent advance in high-resolution light microscopy (stimulated emission depletion microscopy, STED) for the analysis of synapse substructures. STED breaks the diffraction barrier and allows localization of proteins well below 100 nm. Thus, we found that Bruchpilot shapes the presynaptic active zone architecture by adopting an extended conformation (*1, 6, 7*). Now, we are combining STED sub-diffraction resolution with *in vivo* visualization of macromolecular organization.

Our studies promise insights into the proteins architectures organizing synapses in structural and functional terms. In fact, we recently found that mutations in rim-binding protein, a protein which by STED we detect in the active zone core, severely affects synaptic vesicle release from the active zone (*8*). Notably, two human rim-binding protein loci seem associated with autism, a neurodevelopmental disorder characterized by impairments in reciprocal social interaction, communication deficits and repetitive and restricted patterns of behavior and interests.

References:

1. R. J. Kittel *et al.*, *Science* **312**, 1051 (May 19, 2006).
2. D. A. Wagh *et al.*, *Neuron* **49**, 833 (Mar 16, 2006).
3. T. M. Rasse *et al.*, *Nat Neurosci* **8**, 898 (Jul, 2005).
4. P. Fuger, L. B. Behrends, S. Mertel, S. J. Sigrist, T. M. Rasse, *Nat Protoc* **2**, 3285 (2007).
5. A. Schmid *et al.*, *Nat Neurosci* **11**, 659 (Jun, 2008).
6. W. Fouquet *et al.*, *J Cell Biol* **186**, 129 (Jul 13, 2009).
7. D. Owald *et al.*, *J Cell Biol* **188**, 565 (Feb 22).
8. K. S. Liu *et al.*, *Science* **334**, 1565 (Dec 16, 2011).

STUDY OF LOW-FREQUENCY DYNAMICS OF LYSOZYME AT DENATURATION INDUCED BY GUANIDINE HYDROCHLORIDE BY INELASTIC LIGHT AND NEUTRON SCATTERING

A.V. Svanidze[1], J.P. Embs[2], V.P. Romanov[3], S.G. Lushnikov[1]

[1]Ioffe Physical Technical Institute, St. Petersburg, Russia

[2]Paul Scherrer Institute, Villigen, Switzerland

[3]St. Petersburg State University, St. Petersburg, Russia

E-mail: annasvanidze@yahoo.com

Investigation of changes in protein dynamics and structure under the action of different factors attracts considerable attention, since phase transformations of proteins accompany all biochemical processes. Study of protein denaturation mechanisms is of great importance because it sheds light on the stability of proteins and mechanisms of there folding and unfolding. One of small globular proteins - hen egg white lysozyme, has been chosen as a model object for our experiments on denaturation induced by guanidinium chloride (GdnHCl). In molecular biology, GdnHCl is one of the most frequently used substances for the experiments on protein denaturation and refolding. It was demonstrated by molecular dynamic simulations that guanidinium ions (Gdm^+) could form complexes [1], as well as bind to protein amino acid residues [2]. We have studied GdnHCl solutions and solutions of lysozyme denatured in 6 M GdnHCl by Brillouin light scattering and inelastic neutron scattering. The strong quasi-elastic scattering in Brillouin spectra has been revealed for the solutions with high GdnHCl concentration. Taking into account the results of molecular simulations presented [2], it was proposed that the nature of quasi-elastic component is the formation of Gdm^+–Gdm^+ complexes and Gdm^+–protein binding [3]. The analysis of quasi-elastic scattering in the dynamic structure obtained be inelastic neutron scattering have been performed in order to analyze the diffusive motion in the solutions of GdnHCl and solution of lysozyme denatured by 6 M GdnHCl.

References:

[1] P.E. Mason, G.W. Neilson, J.E. Enderby, M.-L. Saboungi, C.E. Dempsey, A.D. MacKerell, Jr. and J.W. Brady, J. Am. Chem. Soc. 2004 (2004) 11462.

[2] P.E. Mason, J.W. Brady, G.W. Neilson, C.E. Dempsey, Biophys. J.: Biophys. Lett. (2007) L04.

[3] A.V. Svanidze, S.G. Lushnikov, V.P. Romanov, S. Kojima, J. Raman Spectrosc. (2012) in press.

MECHANISMS OF THE PEPTIDERGIC REGULATION OF CELL DIFFERENTIATION IN THE PANCREAS: MOLECULAR MODELING *in silico*

Svetlana Tarnovskaya[1], Galina A. Ryshak[1], Pavel Yakutseni[2]

[1]Saint-Petersburg Institute of Bioregulation and Gerontology, Russia

[2]Center for advanced studies, Saint-Petersburg State Polytechnical University, Russia

E-mail: svetlanatarnovskaya@gmail.com

Tetrapeptide Lys-Glu-Asp-Trp was synthesized in the St. Petersburg Institute of Bioregulation and Gerontology as a mimetic of insulinotropic polypeptide. In human pancreatic epithelial cells this tetrapeptide stimulates expression of differentiation factors of β-cells (Pdx1, Pax4, Foxa2, NKx2.2) that lies on the basis of its insulinotropic and antidiabetic effects.

The aim of the research was to create a three-dimensional model of interaction of tetrapeptide Lys-Glu-Asp-Trp with DNA for determination of peptidergic regulation.

A fragment of B-DNA (5'-d[GCTAGCAGTCC]-3'•5'-d[GGACTCGCTAGC]-3') was taken from the PDB database, PDB code 2KV6. An initial PDB code was taken as a first starting point of simulation. Other starting points of B-DNA models were used also. The conformation of tetrapeptide Lys-Glu-Asp-Trp in left handed stereo configuration was calculated in MM+ and Amber99 force fields using molecular dynamics method. The best worked out conformation of Lys-Glu-Asp-Trp was chosen for DNA-peptide complex model. The bond energy of DNA-peptide complex was calculated in Amber99 force field as the difference between energy of separate DNA and tetrapeptide and energy of their complex. The generalized Born model was used for implicit water simulation.

Two models of B-DNA-tetrapeptide complex were created: the first is the interaction of Lys-Glu-Asp-Trp with the minor groove of B-DNA, the second is the interaction of Lys-Glu-Asp-Trp with the major groove of B-DNA. Analysis of molecular mechanics parameters (hydrogen bond, electrostatics, hydrophobicity and bond energy of DNA-peptide complex) showed that more profitable is the second model. In the major groove of DNA tetrapeptide interact by its polar side chains with amino groups of nucleobases; hydrogen bonds were observed between amino group of adenine and main-chain oxygen atom of aspartic acid and between thymine and C-terminal carboxamide group of tetrapeptide. Cation-π interactions were found between nitrogenous cations of tetrapeptide and aromatic rings of nucleobases. The minor groove of DNA in first

model is formed by closely located negatively charged phosphate groups that pushe off polar groups of tetrapeptide. In this case, tetrapeptide interacts with DNA only by its hydrophobic tryptophan which intercalates into DNA from the minor groove.

The structural models, obtained by the molecular modeling, and revealed peculiarities of the interaction of tetrapeptide Lys-Glu-Asp-Trp with 5'-d[GCTAGCAGTCC]-3'•5'-d[GGACTCGCTAGC]-3' sequence confirms the possibility and nature of the formation of complexes of peptides and DNA that means this tetrapeptide can participate in gene expression of differentiation factors of β-cells (Pdx1, Pax4, Foxa2, NKx2.2). A comparison between our model and experimental data is under discussion. The model of B-DNA-tetrapeptide complex explains the mechanism of peptidergic regulation of tetrapeptide in cell differentiation of the pancreas. Further the results of this study can be used in drug design.

DNA-PHOTOSENSITIVE SURFACTANT INTERACTION

Evgenii Titov[1], Svetlana Santer[2] and Nina Kasyanenko[1]

[1]Saint Petersburg State University, Russia

[2]University of Potsdam, Germany

E-mail: evgenii.v.titov@gmail.com

DNA is rigid and highly charged polymer that undergoes strong compaction inside cell nuclei in nature. Many agents such as multivalent ions, polycations, some surfactants and others induce DNA packing in a solution (DNA condensation). DNA condensation is of current interest for the formation of gene vectors in genetic engineering. This process can be also used in nanotechnology. DNA interaction with photosensitive surfactants is of special interest due to the possibility of the influence on a DNA conformation by the light.

Calf thymus DNA (Sigma) was used. Azobenzene trimethylammonium bromide surfactant (AzoTAB) was synthesized at the University of Potsdam. The chemical structure of the surfactant is shown in Figure 1. The Atomic Force Microscopy, AFM (Veeco NanoScope III), Dynamic Light Scattering, DLS (Photocor Complex), Low-gradient Viscometry, Spectrophotometry (SF-56, SF-2000) techniques were used.

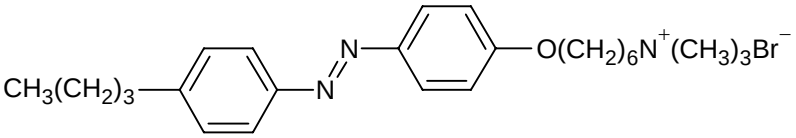


Figure 1. Chemical structure of AzoTAB.

AzoTAB undergoes a reversible photoisomerization. In the dark the surfactant is mainly in the trans conformation. After UV light illumination (the wavelength of 365 nm) AzoTAB converts to the cis conformation. The cis-trans transition can be achieved in the dark or with visible light illumination (the wavelength of 453 nm).

It was shown that NaCl concentration in an aqueous AzoTAB solution influences on the critical micelle concentration (CMC). The hydrodynamic radius of AzoTAB micelles in 5 mM NaCl R_h = 3 nm was determined by the DLS technique at 5 mM AzoTAB concentration.

The viscometry data reveal that DNA compaction by AzoTAB occurs at low NaCl concentration (5 mM) and is not observed in 1 M NaCl. The reduced viscosity of the DNA-surfactant solution decreases in 5 mM NaCl with the increase in charge ratio Z, defined as [AzoTAB], M / [DNA], M (nucleotides). The precipitation is visually

observed for $1.0 \leq Z \leq 1.8$ at [DNA] = $2.5 \cdot 10^{-4}$ M (nucleotides). The turbidity is observed for $Z > 1.8$ without visible precipitation when the reduced viscosity of the DNA-surfactant solution is close to zero. It should be noted that the wide range of AzoTAB concentrations was used during the experiment (less and more than CMC — for AzoTAB in 5 mM NaCl in the dark or with visible light illumination (453 nm) CMC is around $1.5 \cdot 10^{-4}$ M). It was shown that UV light illumination (365 nm) of the samples with $Z = 2.0$ and $Z = 3.1$ induces the precipitation (like for $1.0 \leq Z \leq 1.8$).

The DLS measurements indicate that for the DNA-AzoTAB solution with $Z = 0.2$ the particles with the hydrodynamic radius of around 40 nm are presented. It is interesting to note that the reduced viscosity of the solution with $Z = 0.2$ is less than for the DNA solution in the absence of the surfactant but not too low. One can assume that there is the coexistence of DNA extended coils and compacted DNA molecules in the certain range of Z values.

The AFM images of DNA and DNA-AzoTAB complexes are presented in Figure 2 and Figure 3 respectively.

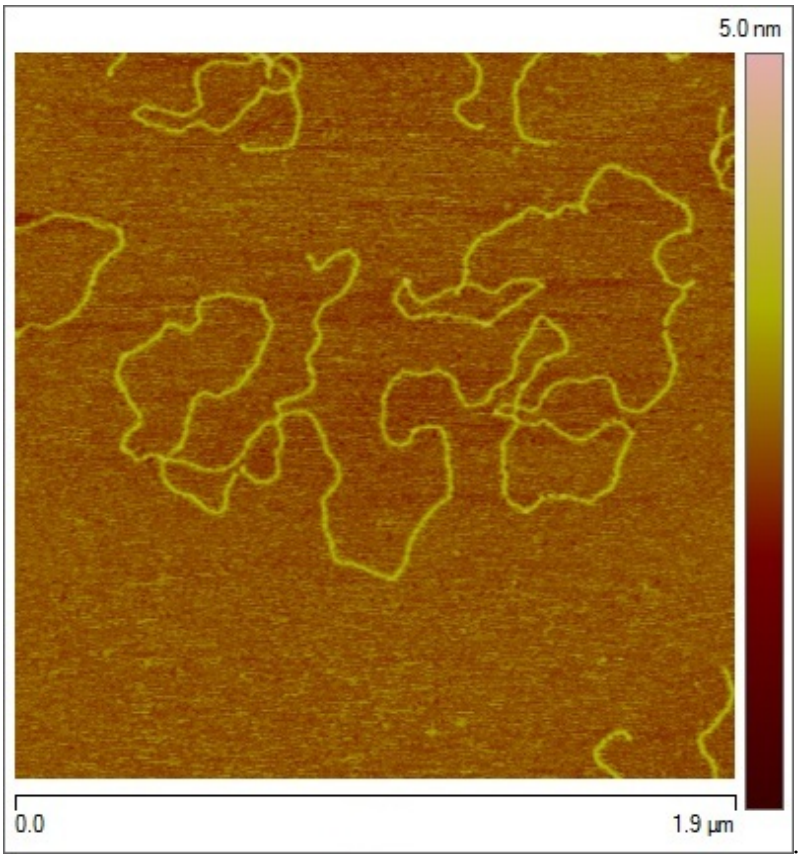


Figure 2. AFM image of DNA.

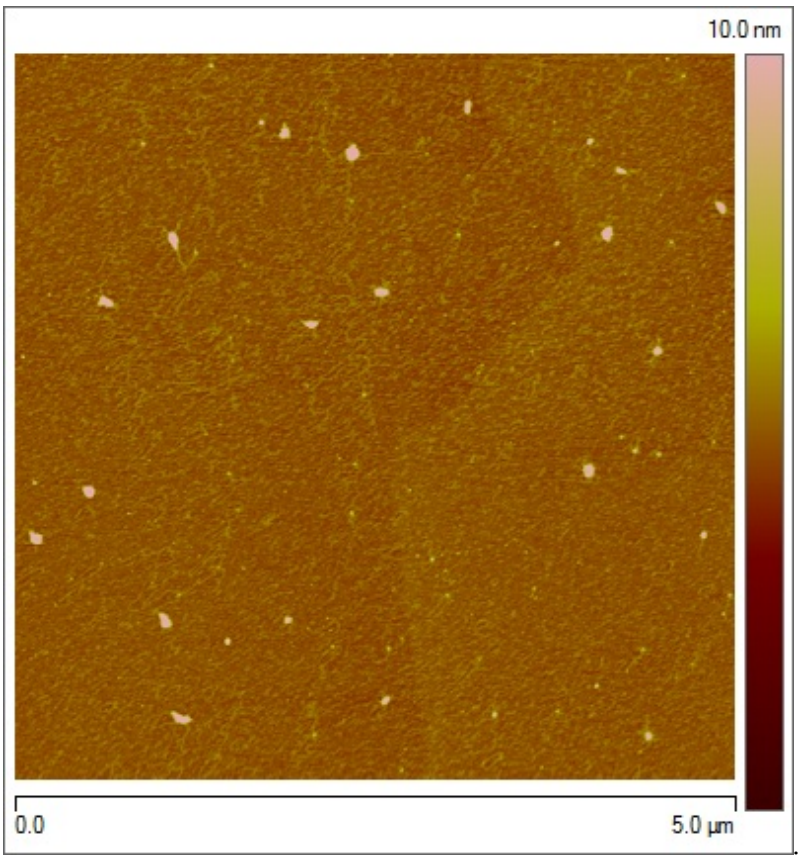


Figure 3. AFM image of DNA-AzoTAB complexes.

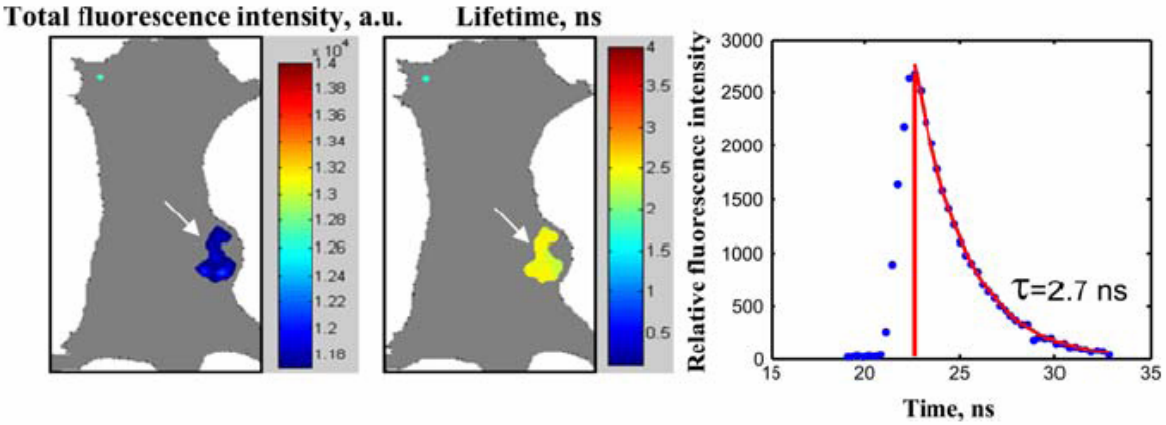
FLUORESCENCE AND PHOTOACOUSTIC SMALL-ANIMAL *in vivo* IMAGING

Ilya Turchin

Institute of Applied Physics of the Russian Academy of Science
Nizhny Novgorod, Russia
E-mail: ilya@ufp.appl.sci-nnov.ru

Optical techniques are very attractive for small-animal imaging in application to different research studies provided in oncology, pharmacology, embryology and neuroscience [1]. Among them fluorescence (FLI) and photoacoustic (PA) imaging modalities are the most rapidly emerging technologies to non-invasively follow all kinds of molecular and cellular processes in small animals.

Fluorescence imaging techniques enable noninvasive real-time whole-body visualization of fluorophores in small animals. A number of fluorophores, such as organic dyes, quantum dots, fluorescent proteins, and photosensitizers have been imaged in small animals in vivo. Among the fluorescence imaging techniques, fluorescence diffuse tomography is definitely a more advanced technique, enabling 3-D volumetric imaging [2] of fluorescent agents in deep tissues with a resolution of 1 to 2 mm. Nontomographic imaging, both planar reflectance and transillumination modality, is considered to be an alternative approach for fluorescence detection in living organisms [3]. Planar reflectance imaging or epi-illumination is the most widely exploited but its application is restricted to observation of superficial lesions. The transillumination method enables 2D deep-tissue imaging. Being technically easy to implement and simple in operation in comparison with fluorescence tomography, it is an attractive tool for deep fluorescence imaging [4]. There are several promising directions of fluorescent imaging evolution, one of them is the whole-body time-resolved fluorescence lifetime imaging (FLIM) which allows one to estimate kinetics of the fluorophore decay [5].



In vivo whole-body lifetime imaging of subcutaneous TurboRFP-expressed tumors in nude mouse. The graph shows an example of the approximation of the fluorescence intensity decay in the tumor area and the calculated lifetime [5].

Photoacoustic (PA) imaging is a modern method of biomedical visualization based on recording of ultrasonic waves excited in the investigated medium due to absorption of pulsed laser radiation by optical inhomogeneities [6]. The main advantage of the PA imaging techniques compared with the purely optical methods is improved spatial resolution at depths from a few millimeters to a few centimeters. Modern pulsed lasers permit wavelength tuning to achieve the maximum gradient of optical absorption of investigated structures with respect to the surrounding tissues. Therefore, it is possible to optimize PA contrast of endogenous light-absorbing agents (such as hemoglobin, melanin, water, etc.), which permits one to visualize the vascular pattern of tissues and to determine the local tissue oxygenation status, the rate of blood circulation, and the local internal temperature [7]. The use of exogenous contrast markers (organic dyes, nanoparticles, fluorescent proteins, reporter genes, etc.) provides molecular imaging [8] with enhanced contrast, which is unachievable by the conventional bioimaging techniques such as ultrasonography.

References:

1. SR Cherry, "In vivo molecular and genomic imaging: new challenges for imaging physics", Phys. Med. Biol. 49(3), pp. R13-R48 (2004)

2. V. Ntziachristos, J. Ripoll, L.Wang, R. Weissleder, "Looking and Listening to light: the evolution of whole-body photonic imaging", *Nature Biotechnology*, **23**, 313-320 (2005).

3. V. Ntziachristos, G. Turner, J. Dunham, S. Windsor, A. Soubret, J. Ripoll, and H. A. Shih, "Planar fluorescence imaging using normalized data," *J. Biomed. Opt.* 10, 064007 (2005).

4. Marina Shirmanova, Elena Zagaynova, Marina Sirotkina, Ludmila Snopova, Irina Balalaeva, Irina Krutova, Nataliya Lekanova, Ilya Turchin, Anna Orlova, Michail Kleshnin, "In vivo study of photosensitizers pharmacokinetics by fluorescence transillumination imaging", // J. Biomed. Opt., Vol. 15, 048004 (2010)

5. Alexander L. Rusanov, Tatiana V. Ivashina, Leonid M. Vinokurov, Ilya I. Fiks, Anna G. Orlova, Ilya V. Turchin, Alexander P. Savitsky Life-time mode of FRET measurements for red fluorescent proteins. Journal of Biophotonics, 3(12), p. 774–783 (2010).

6. T. D. Khokhlova, I. M. Pelivanov, A. A. Karabutov, " Methods of optoacoustic diagnostics of biological tissues", Acoustical Physics 55, 674 (2009).

7. P. Beard, "Biomedical photoacoustic imaging", Interface Focus 1, 602 (2011).

8. L.-H. V. Wang, S. Hu, "Photoacoustic tomography: in vivo imaging from organelles to organs", Science 335, 1458 (2012).

MODEL STUDY OF THE BIOLOGICAL EFFECTS OF MAGNETIC FIELD: ANTIBACTERIAL ACTIVITY OF TOTAL THYMUS HISTONE IN ATTENUATED GEOMAGNETIC FIELD

Vladimir Yukhnev[1,2], Vladimir Kokryakov[1,2], Boris Shchegolev[3], Vasily Stefanov[1]

[1] St. Petersburg State University, Biology-Soil Faculty

[2] Institute of Experimental Medicine RAMS

[3] Pavlov Institute of Physiology RAS; Russia, St. Petersburg.

E-mail: yuk-vladimir@yandex.ru

Endogenous antimicrobial peptides and proteins of humans and animals play a key role in the implementation and regulation of innate immunity, providing immediate anti-infectious protection for organisms. Antimicrobial peptides (defensins, katelitsidiny, etc.) and proteins (histones, lysozyme, serprotsidiny, lactoferrin, peroxidase) of animals have a direct microbicidal activity against a broad range of microorganisms (bacteria, fungi, protozoa, enveloped viruses), and in some cases appear as an immunomodulatory molecules.

Increased resistance of microbes to the conventional antibiotics (penicillin, gramicidin, etc.) involves the problem of search and study of alternative antimicrobial substances with antibiotic and immunomodulatory activity, without the development of resistance by bacteria. One such approach is to search, selection, and structural and functional analysis of polypeptide antibiotics of animal origin, which are natural components of the molecular mechanisms of anti-infective protection of humans and animals. In addition, the discovery of the most effective antimicrobial polypeptide antibiotics dictates the need to find non-invasive ways to enhance their antibiotic action. A promising direction of research in this area is to study the effects of antimicrobial substances of animal origin in weak magnetic fields (WMF). The impact of such fields can cause changes in the morphological and physiological characteristics of microbial cells and thus modulate their sensitivity to anti-infective polypeptide agents.

The aim of the work was to study the antimicrobial activity of the total fraction of calf thymus histones in the conditions of weak magnetic fields of Earth (WMFE). By method of 'radial diffusion' we were carried out two series of antimicrobial tests. As endogenous antimicrobial compounds used preparation of thymus total histone (TTH). Testing was conducted against the Gram-positive (Listeria monocytogenes EGD) and Gram-negative (Escherichia coli ML 35p) bacteria. Experiments were conducted under conditions of normal MFE at different implementation stages of antimicrobial test. To

weaken the Earth's magnetic field was made a closed cylindrical chamber with a shielding cover, which is a tube of length L = 31cm and the diameter D = 10 cm, which are coiled several layers of amorphous magnetic material AMAG-172. The measurement of the magnetic field in the "working volume" chamber were carried out using three-component magnetometer HB0302.1A (0.1-100 mT). The geomagnetic field at the site of the experiments (48mkTl) in the chamber was weakened by 90 times. As control served test conducted outside the chamber. In the first phase of work was done testing of antimicrobial activity of TTH for micro-organisms grown under normal conditions at 37 º within 18-20 hours. After three hours of microbes growth experiment was conducted in three versions: a Petri dish with the test organisms in agarose did not put in a cell, all subsequent experiments were carried out in the chamber, placed in the chamber after a 3-hour incubation of bacteria with proteins. It is established that the action of TTH on the microbes in the conditions of weak magnetic fields of Earth on both E. coli and Listeria monocytogenes observed the effect of increasing their antibiotic action on ~ 50-75% compared with control options. In the second series of experiments was tested the viability and sensitivity of Gram-negative E. coli to the effects of TTH in WMFE, from the stage of night growing microbial cultures. After microbes growth to the exponential phase the experiment was carried out as in the first series in three versions. It is shown that compared with the first series of experiments we observed a more pronounced enhancement of antimicrobial properties of TTH (two times in all variants of the experiment).

Thus, the phenomenon is set to enhance the antimicrobial action of TTH in terms of weak magnetic fields, especially in the variants of microbes that growth in WMF. Explanation of possible structural and functional changes that underlie the established phenomenon, will be the subject of our further research.

Supported by grant of Russian Foundation for Fundamental Research (12-04-01737), Federal special-purpose program «Scientific and educational personnel of innovation Russia» (2009-2013) and grant of St. Petersburg State University (project No. 1.0.130.2010).

INFLUENCE OF MUTATIONS IN *Drosophila agnostic* LOCUS ON STRUCTURE AND CONFORMATIONAL STATE OF *limk1* DNA SEQUENCE

Alexander Zhuravlev*, Anna Medvedeva*[1], Elena Savvateeva-Popova*[1], Sergey Lushnikov.**

*Pavlov Institute of Physiology, RAS
[1]St. Petersburg State University
**Ioffe Physical Technical Institute, RAS
E-mail: beneor@mail.ru

LIM-kinase 1 (LIMK1) regulates the neuronal actin remodeling signal cascade via inhibition of actin-depolymerization factor cofilin thereby affecting synaptic neuroplasticity. The *limk1* mutants of *D. melanogaster* demonstrate the multiple physiological and behavioral features: the changes of temperature sensitivity, learning and memory formation, sound production, locomotor activity, and others. *limk1* is localized within the *agnostic* locus (X: 11AB) in the region with the high frequency of spontaneous rearrangements. However, the molecular mechanisms of *limk1* activity disregulation are still unknown. Sequencing of *limk1* DNA has been carried out for *agnostic* mutant agn^{ts3}obtained in our laboratory, and also for three wild-type strains with different behavioral activity - *Canton-S (CS)*, *Oregon-R (OrR)* and *Berlin (Ber)*. Surprisingly, we revealed some common structural features for agn^{ts3} and *OrR*. Among them are several small deletions and insertions in intron 1, including 28 bp AT-rich insertion, le of forming the cruziform structure. The major part of one-nucleotide substitutions in agn^{ts3} and *OrR* seem to result from the fixation of one allele of ancestor heterozygous strain: *CS* - for agn^{ts3} and in part for *OrR*. The site of M1 transcription binding factor in both strains carries a substitution that may decrease its affinity to DNA. The 15 bp insertion should lead to the additional 5 amino acid inclusion into the C-terminal protein kinase domain of *OrR* LIMK1. *Ber* sequence is the most similar to *CS one*, both carrying the polymorphic nucleotides in the positions of *OrR* and agn^{ts3} substitutions**.** The region following the 3'-end of agn^{ts3} *limk1* carries an insertion of the natural trasposone of Tc1/mariner family, which may affect the gene expression. The temperature influences the model structures of DNA hairpins near the 28 bp insertion which may cause the temperature-dependent changes of LIMK1 level. Taken together these data reveal the possible mechanisms for LIMK1 activity disregulation in both the mutant and the wild-type strains.

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DNA–METALLIC NANOPARTICLES SYSTEMS

R. A. Belykh, M. S. Varshavskiy, V. I. Rolich, N. A. Kasyanenko

Department of Molecular Biophysics
Faculty of Physics
Saint-Petersburg State University
E-mail: varshavskiimiha@mail.ru

Metallic nanoparticles (NPs) nowadays are the objects of intense study due to the rapid development of new nanomaterials. Their unique size-dependent properties make these materials attractive in many areas of high technologies. E.g. sensors for applications in medicine and biology, optoelectronic nanodevices in which nanometer size of the individual elements are related to optical frequencies they operate.

Silver and aluminum NPs investigated in this work were synthesized in NTU of Ukraine "Kyiv Polytechnic Institute" with electric-spark erosion technique [1]. Silver NPs synthesized chemically in our lab were also studied. In the experiments we used calf thymus DNA (Sigma) with a molecular weight of $8 \cdot 10^6$ Da, polyallylamine (25 000 Da), $NaBH_4$, $AgNO_3$, NaCl, $MgCl_2$, $AlCl_3$.

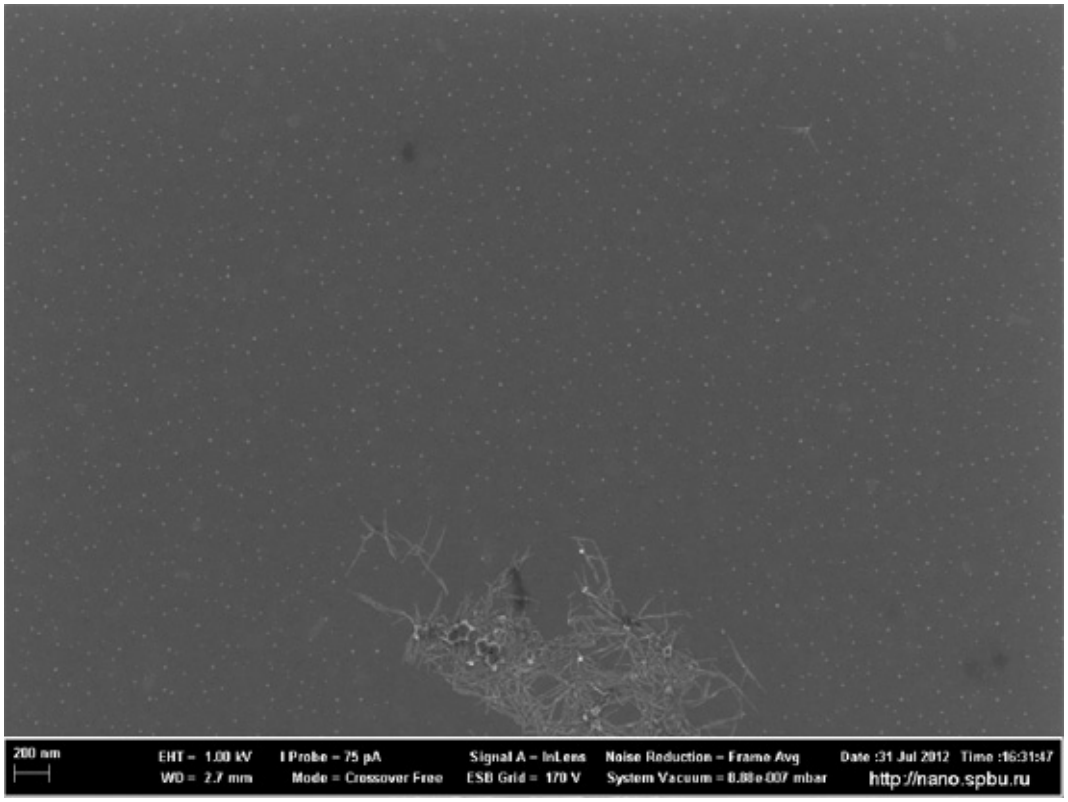
The methods used were UV adsorbance and Cirsular Dichroism spectroscopy, viscometry, Atomic force microscopy and Scanning Electron Microscopy.

It was shown that silver nanoparticles are stable in aqueous solution. The presence of DNA in solution influences the silver nanoparticles and stabilizes the system. The optimal ratio of silver nitrate and sodium borohydride for nanoparticle synthesis in aqueous solution was determined. The nanoparticles acquired are stable for several months and have an average size of about 12 nm.

The Al NPs solution investigated contained the particles of two types, "needle-like" structures with length of 200–300 nm and thickness of 2–3 nm, and spherical particles with diameter of 10–80 nm. It was shown that spherical ones bind to DNA in solution, and cause it's precipitation. When emplaced onto silicone surface the precipitate complexes form a regular net of DNA wisps with a nanoparticle set in every crossing.

References:

6. А.А. Щерба, С.Н. Захарченко, К.Г. Лопатько, Н.И. Шевченко, М.О. Ломко. Разрядно-импульсные системы производства наноколлоидных растворов биологически активных металлов методом объемного электроискрового диспергирования. Праці ІЕД НАНУ, Вип. 26, 2010.



SEM image of sedimented precipitate of DNA–Al NPs complex droplet dried on silicone layer.

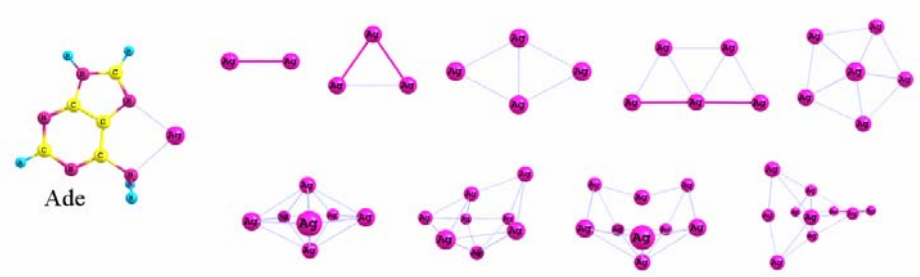
PHOTOREDUCTION OF SILVER IONS ON THE ADENINE

R. R. Ramazanov, A.I. Kononov, N. A. Kasyanenko

St.Petersburg State University
E-*mail: r.ramazanov@spbu.ru*

Creation of various luminescent bioprobs have been a challenging task for biomedicine diagnostic of diseases. Chemical reduction of silver nanoclusters on DNA as a stabilizing matrix allows to obtain luminescent structures with a high quantum yield. According to the one of the standard procedures of receiving such structures the growth of clusters is realized due to the interaction of positive silver ions with the nitrogenous bases of DNA containing electronegative lone pairs of electrons on the nitrogen. Further addition of a reducing agent into DNA solution leads to the appearance of metal atoms of silver, which might interact with silver ions on DNA with subsequent formation of metal complexes (clusters). The clusters grow till a certain size - the geometrical parameters of clusters can be defined by the secondary structure and base sequence of DNA. The multicomponent system including DNA, silver salt and reducing agent determine an intricate picture of chemical processes. In this regard the problem of creation of silver nanoclusters with required optical characteristics is very complicated. In this work theoretical research of possibility of silver ions photoreduction on DNA nitrogenous bases instead of chemical agents is carried out.

The system «adenine-$Ag^{+}(Ag)_{n}$» in vacuum, where n=0-10 was considered. Also free nanoclusters of $Ag^{+}(Ag)_{n}$, where n=0-10 was considered for the verification of the chosen modeling approach. Quantum chemical optimization of molecular geometry of complexes, calculation of excitation spectra and optimization of excite state were carried out in "supermolecule" approach within the density functional theory with use of a hybrid functional PBE0. Ag atom was represented through a LANL2DZ relativistic effective core potential RECP and considered as a Ag19+ core. The other atoms were represented through a valence double-zeta polarized basis set 6-31g*. For the optical part, the calculation of the properties (excitation energies, oscillator strengths, and transition dipole moments have been carried out in the framework of TDDFT implemented in Gamess US with the time-dependent Kohn–Sham formalism and related techniques. The figure demonstrates different silver clusters as a result of calculation.



Calculation of excitation spectra for a various set of free silver clusters showed a good agreement with literature data for the chosen approach. In an equilibrium configuration the length of bond Ag^{+}-N7 (adenine) for different clusters (2,30±0,06)Å, and length of bond Ag-Ag - (2,75±0,08)Å remain almost identical. Optimization of the excited state (transition S0-S1) leads to the disintegration of the complex adenine + $Ag^{+}(Ag)_n$ for n <3. The distance between the Ag^{+} and N7 (adenine) is greater than 4Å. In this case charge transfer from the adenine to the silver cluster causes a reduction of positive silver ion. For n > 3 charge transfer and change of symmetry of silver clusters is also observed, but dissociation of the complex does not occur through the redistribution of the charge over all cluster atoms. This fact allows photoreduction of silver ions bound with adenine for small atom groups with n <3. Such silver metal atoms and atomic groups can serve as initial building blocks for the further growth of large clusters.

DNA-BASED FLUORESCENT SILVER NANOCLUSTERS

Ivan Volkov, Alexey Misorin, Alexey Kononov and Nina Kasyanenko

Saint-Petersburg State University
Physics faculty, Department of molecular biophysics
E-mail: i.volkov@spbu.ru

Silver nanoclusters are agglomerates of few to tens silver atoms, probably in partially oxidized form $Ag_nAg_m^{m+}$. Because of extremely small size and suitable electronic levels configuration they have a luminescent properties with strong emission in visible and infra-red range. Nowadays they attract intense scientific interest due to their stable luminescence and very simple way of synthesis. They was made and explored in argon matrix earlier, but new approaches of stabilizing Ag-clusters by polyanions allow synthesizing and using them in aqua solutions. Silver clusters made on the base of biomolecules have great advantages over well known semiconductor quantum dots or organic dyes because of the small size and potential biocompatibility. For instance, DNA-stabilized luminescent silver nanoclusters were made lately on the base of oligonucleotides for bio-labeling purposes. Regardless of the wide applied research, the fundamental mechanisms of silver clusters growing are still poorly understood.

In this report we present the method of formation of DNA-stabilized silver nanoclusters on the base of high-molecular DNA. This strategy gives us not only the possibility to explore the clusters properties and their formation mechanism but also let us tune the conditions for label-free DNA fluorescent staining *in vivo* in the future.

For sample preparation, we used calf thymus DNA (Sigma). DNA water solution contained 0.005 M $NaNO_3$ to screen phosphates groups and stabilize DNA. $AgNO_3$ salt was added to the solution and DNA-Ag^+ complexes were stored for one day. Then reducing agent $NaBH_4$ was added to the solution to produce nanoclusters growing. All manipulations were performed at 21° C temperature.

Fluorescent analysis showed a large variety in luminescence of clusters those emission bands are in the range of 530–730 nm. This fact can be explained in connection with the literature data showing that luminescent properties of silver nanoclusters strictly depend on DNA-sequences, and those are very different for thymus DNA. Excitation spectra have 2 major bands in UV (270 nm) and visible region (500-700 nm). According to the polarization of luminescence data we can conclude that luminescent clusters are connected to DNA bases indeed and the energy transfer from excited nitrogen base to silver cluster are realized.

We also demonstrate the condensation of DNA filled with silver luminescent clusters by polycation (polyallylamine). This approach allows producing compact (~100nm) luminescent globules keeping fluorescence of silver clusters connected to DNA.