UDC 576.851.49/ 57.083.1

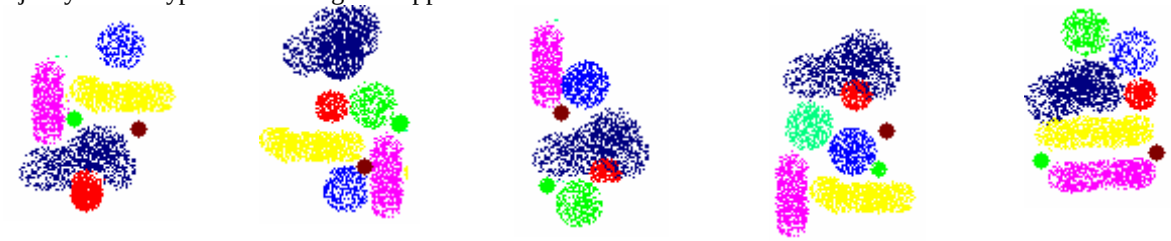
NEW APPROACH TO DESIGN AND SYNTHESIS OF THERAPEUTIC AND PREVENTIVE DRUGS, TAKING INTO ACCOUNT INTERSPECIES POLYMORPHISM OF RECEPTORS (METHOD OF PRECISION PARTIAL MODIFICATION)

Martynov A.V., Smelyanskaya M.V., Peremot S.D.

Mechnicov Institute of microbiology and immunology of UAMS

New Approach to Development and Synthesis of Therapeutic and Preventive Drugs, Taking into Account Interspecies Polymorphism of Receptors (Method of Precision Partial Modification)

An important place in the modern structure of the pharmaceutical market belongs to drugs – biotechnology products of various origins. These are such important drugs for patients' lives as recombinant insulins, interferons, interleukines, erythropoietins, etc. At the same time, statistic data of bringing such drugs, as well as a number of low-molecular drugs, into the market show a little effectiveness percentage excess over placebo. For example, an effectiveness percentage excess of beta-interferon (in the treatment of disseminated sclerosis) over placebo is only 8 % (30% of placebo and 38% of beta-interferon) [1]. The situation is almost the same for low-molecular drugs, for example, antihypertensive. Effectiveness of amlodipin at the third stage of clinical tests was only 22 % higher than of placebo: 52% to 30% of placebo) [2,3]. Causes of the fact that a drug turns out to be ineffective or of little effect for 48% of patients have not been found out yet. The most difficult to explain is ineffectiveness of drugs, whose acceptors are cell receptors studied long ago, namely, adreno-, cholino- and histamine receptors. Absolute ineffectiveness of one and the same drug for one group of patients at its effectiveness in the other one remains a mystery. Due to this little studied peculiarity of a human organism the majority of antihypertensive drugs are applied in combination. It is especially necessary to combine at least three drugs having different mechanisms of action but the same final result: for example, antihypertensive or cytostatic. In the latter case there is the brightest expression of differences between various kinds of tumors not only by sensitivity, but also by individual peculiarities of a specific tumor and host organism. Even polychemotherapy often turns out to be ineffective in the treatment of patients with cancer. Depressing statistic data of FDA concerning the third stage of clinical tests of drugs show low effectiveness of practically all medicinal drugs available in the pharmaceutical market. Average effectiveness of the strongest drug (morphine) is 75%. In other cases we observe either intolerance and toxic effects or an opposite action. Even in cases of applying narcotic drugs only 60 % of people, who took them, have classical effects observed [4]. The other group of people, who took, for example, cocaine, suffer a severe headache and dizziness without any signs of anesthesia [4]. Such divergence of effects may be caused by polymorphism of a receptor system within human population. Earlier, the structure of receptors was considered to be absolutely conservative and invariable for one and sometimes several animal species. At present more and more scientists are inclining to an opinion that receptors differ like human faces even within one species. These differences are caused not only and not so much by the change of the primary amino acid sequence of receptors protein base as by conformation changes of secondary and tertiary structures [5]. Having formal similarity in the primary structure and molecular weight, receptors of different people are actually combinations of proteins isoforms. It is especially well-seen in the example of major histocompatibility complex (MHC) antigen isoforms combination. Selection of this complex is vitally important at organ transplantation [6]. If there exist thousands of variants and combinations for MHC system, why should structures of other receptors of the organism be conservative within a species? Most likely, similarly to MHC antigens tertiary structures of the majority of cell receptors are significantly different by an isoform profile within a population [7].



MHC B7AB MHC B2AE MHC B4AC MHC B1AL MHC B3AC

Fig. 1. Major histocompatibility complex consists of 7 or 9 monomers, the pictures show protein sections on complexes. One and the same proteins are located in different places of the complex and create a unique original structure for each individual. Some people may not have these or those antigens (proteins), this factor also changes the complex architectonics and its antigenicity.

This hypothesis well explains low effectiveness of drugs. A conservative structure of a classical drug (like one "key") cannot match a specific receptor ("many different, though similar locks") of all individuals of one species equally and with equal affinity [8,9].

In order to increase drug effectiveness it is necessary to change the concept of drug development and approaches to this process. For example, docking, [10] one of the most effective methods of modern drug design [11,12], uses a conservative sequence of one receptor. Sometimes target conformations are used in different solvents with

further overlay of conformations. It will never be possible to obtain infinitely many receptors and find ONE inhibitor substance for all conformations. One of successful solutions of nature is immunoglobulin system evolution [13,14] to protect higher organisms from external aggressive factors – viruses, microorganisms and fungi. Practically the same immunoglobulin base (Fc- areas and heavy chains) at a large number of different variants on FAB – fragments being specific to their targets [15] is a quite successful solution.

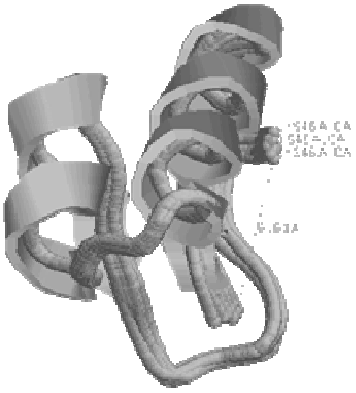


Fig. 2. Overlay of several viscotoxin's molecules (lectin from white mistletoe) onto each other, which were obtained in different conditions.

In this situation affinity to targets may vary from 5% of IgM to 95% of IgG. Sometimes one target antigen may cause generation of several hundred thousand variants of immunoglobulins with different monoclonal specificity for different epitopes. Such polymorphism justifies itself – the majority of the population survives after infectious diseases. And in many cases donor's immunoglobulins of reconvalescents are still the only effective means of treating many diseases, for example, SARS and Marburg fever [16].

Following the logic of nature we realize that choosing the classical way – synthesizing ONE compound to treat one disease is irrational and ineffective. It is proved by decreasing amount of drugs with original structure, which are introduced in the world every year.

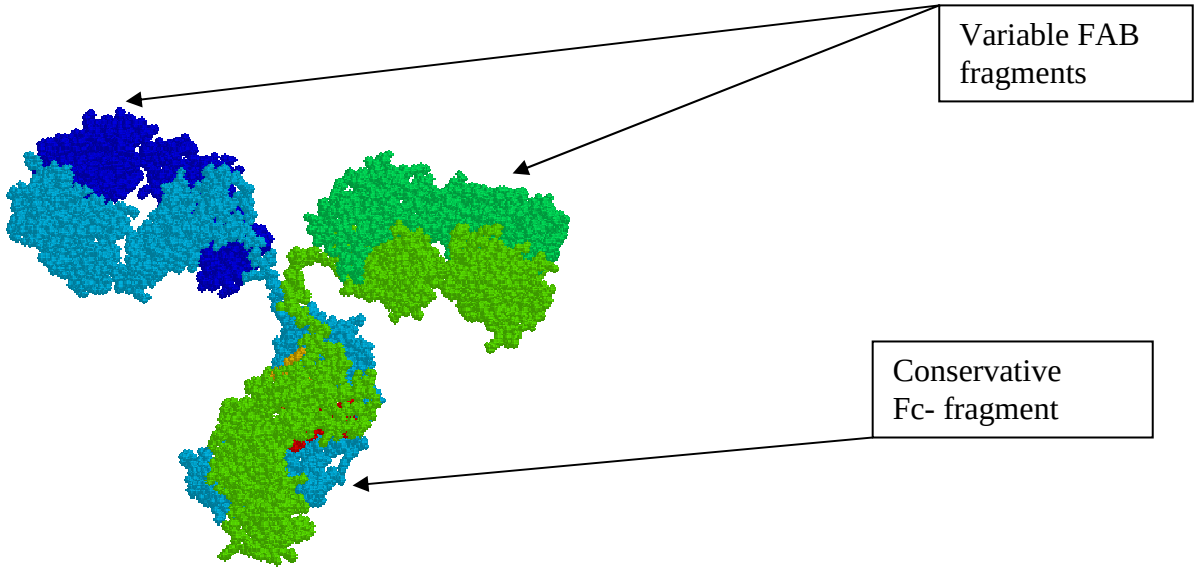


Fig.3. G1 immunoglobulin structure

To provide maximum affinity for maximum number of people it is necessary to have a mixture of millions of molecules very similar but still different from each other in one vial. In this case we obtain not one “key”, but a whole bunch of keys.

And at least one “key” from this “bunch” will match a concrete patient with his/her original receptor. If it is practically unreal in modern conditions to synthesize a concrete inhibitor for a concrete patient, then the only variant is to obtain millions of inhibitor isoforms in one mole of substance.

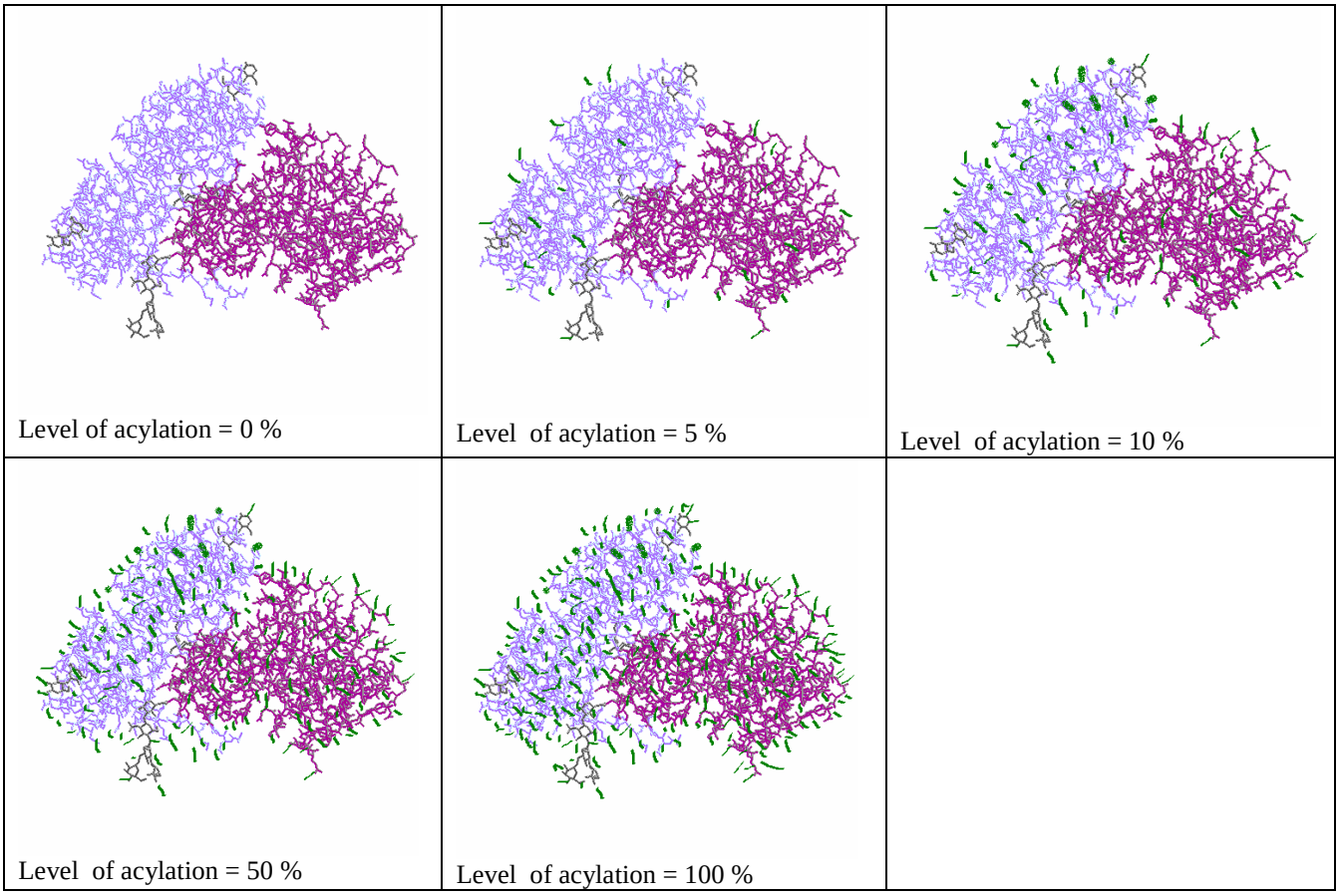


Fig. 4. Ricin, an example of different substituted derivatives combination in one volume of solution from non-substituted to completely substituted derivatives (remains of succinic acid are marked green) in different combinations. At 200 donor groups available for acylation the quantity of variants at 30 % substitution will make 200^{60}. At least one compound from this variety will match the receptor.

One of the most reasonable methods of solving the problem of low effectiveness of drugs in our opinion is obtaining precision partially chemically modified recombinant biotechnological preparations – biopolymers (proteins, polysaccharides, polynucleotides, tannins, self-organized structures – phospholipids, etc.) [17,18,19,20]. The usage of this technology will allow bringing the pharmaceutical science to a level of intensive development, will make molecular modeling methods much more simple and increase probability of practical way out with introduction of drugs.

Molecular Modeling within the Given Concept

One of the main problems in modern molecular modeling is absolute unpredictability both physicochemical and biological properties of compounds designed. As a rule, in addition to the primary structure of compounds an important part is played by stereochemical properties [21]. Here laws of chance take effect. For example, levomycetin has 2 stereoisomers [22,23]. Only one of them, left-rotating, is active.

A B

Fig.5. Isomers of levomycetin (D – (-) –threo-1-p-Nitrophenil-2 dichloracetylaminopropandiol -1,3 (levomycetin in fig.1 B, different position of a hydroxyl group in the stereoisomer is marked green)

The situation is the same concerning compounds newly designed by means of docking: excellent affinity to target protein in the model and complete activity absence in synthesized compounds [24]. This is caused by the fact that ideal conditions of modeling do not take into account the whole variety of influences on the drug-target interaction process. These are such parameters as temperature, character and properties of solvent, presence of mediator additives, gravity, pressure and, of course, conformation of substance [25]. It is the stereochemical structure of designed compounds, which does not coincide with the structure of synthesized compounds in most cases. Synthesis of the necessary substance is often impossible. To eliminate wrong conformations in the process of modeling the compounds with structures confirmed by physicochemical methods should be used as reactants. A contradiction arises again – will such a substance known for a long time be able to inhibit a new target? "A constructor" – proteins and polynucleotides – may be used for this purpose [26].

Correspondingly, if we look for a drug among peptides and polynucleotides in advance and just sort out a monomer sequence, we can find the necessary inhibitor or activator with a high degree of probability and also obtain its synthetic derivative.

The first consist of L- amino acids studied long ago, and the second consist of mononucleotides. Monomer structures are well studied and confirmed, the same as their structures within a polymer and influence of neighboring monomers on each other (this fact is taken into account and used in the process of design and synthesis of primers for a polymerase chain reaction).

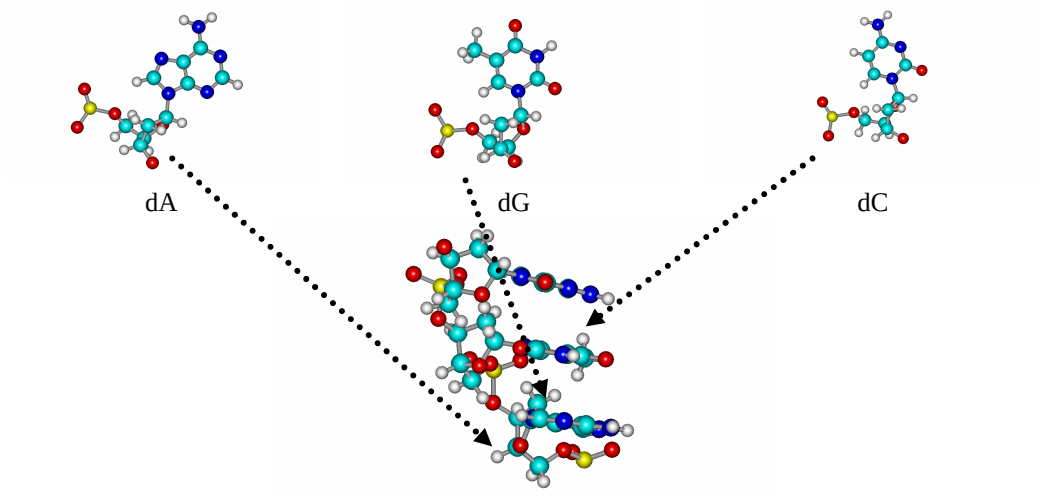


Fig.6. Separate nucleosides are integrated into a polynucleotide as monomer units

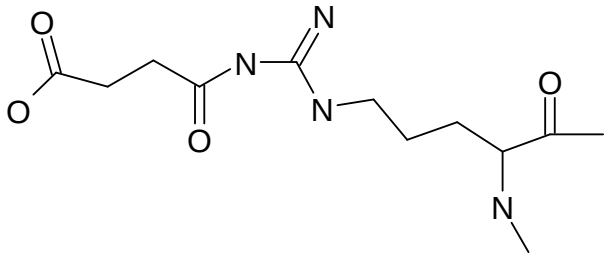
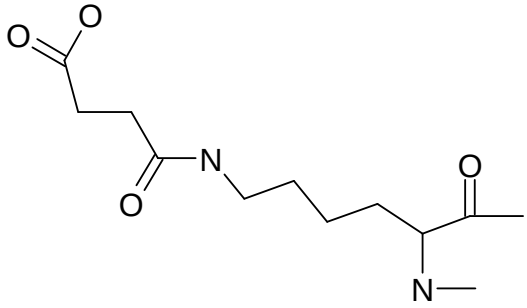
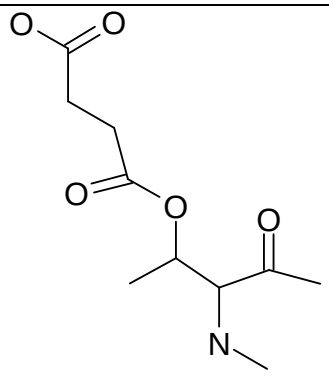
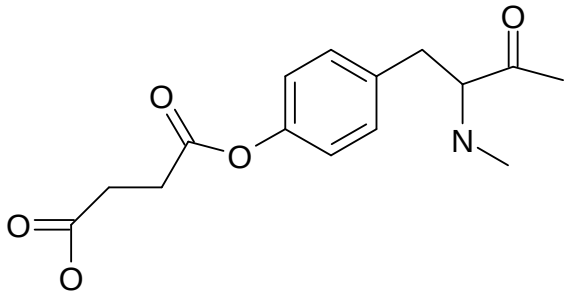
In this situation a number of problem arises: how to vary hydrophobicity and charges of obtained oligomers and how to protect them from the action of lytic factors in the organism – peptidases and nucleases. Besides, these compounds must not be of big size; otherwise they will not be able to get into cells and tissues. One of protein structure modification methods for their better crystallization and studying X-ray structures is acylation of terminal amino groups, lysines and histidines, by means of various agents, among which the simplest are anhydrides of carboxylic (and polycarboxylic) acids – acetic, succinic, maleic acids [27].

The greatest amount of protein crystals studied with the help of X-ray structure analysis method represent completely succinylated derivatives [28].

Succinylation leads to a molecule charge change through substitution of positively charged amino groups by negatively charged carboxylic groups [29]. If acylation

is applied instead of succinylation, not only molecule charge but also molecule hydrophobicity level will change.

Table 1. Structures of modified monomer amino acids, which are formed during protein acylation by means of succinic anhydride

№	Monomer structure	Monomer name
1	2	3
1		Agrinin succinamide
2		Lysine succinamide
3		Ether of threonine and succinic acid
4		Ether of tyrosine and succinic acid

Conformation changes of all amino acids in the acylation process by means of succinic and acetic anhydrides are quite well studied; this fact excludes probable accidents happening during drug design. In this case approaches to modeling agonists (activators) and antagonists are absolutely different taking into account receptors polymorphism, this also concerns vaccine design.

Modeling of Agonists (partially modified interferon, interleukine, immunoglobulins)

Average effectiveness of alpha-interferon in treating, for example, a cervical carcinoma is rather low (about 15 %) [30]. It is connected with the fact that the drug is not able to interact with a cell receptor. In some cases this receptor is blocked by external factors, in other cases it just differs from an "ideal one". Correspondingly, it is necessary to obtain that very "bunch of keys" instead of "one key" – alpha-interferon with a well-defined structure. It may be done by means of partial modification of the same interferon structure. For example, native alpha-interferon contains 8 remains of lysine and three remains of histidine, which are able to be acylated by means of succinic anhydride [17]. In order to get "a set of keys" it is needed to obtain a mixture of maximum amount of various interferon derivatives in one volume with an insignificantly changed active center (the area of connecting to the recep-

tor): from completely acylated to single-substituted in a different combination. This requires selection of a substitution degree, which would provide maximum high activity of the drug and maximum quantity of different molecules in one mole of substance. For alpha-interferon it is a trisubstituted polymorphous derivative. We meaningly used a term " polymorphous " in connection with the fact that actually the solvent contains 3^8 derivatives, as well as little amount of completely substituted and monosubstituted derivatives and the mixture of derivatives with a substitution degree of more and less than three. Thus, partial precision acylation allows obtaining "the set of keys" to one polymorphous receptor and sharply increase drug effectiveness in a population.

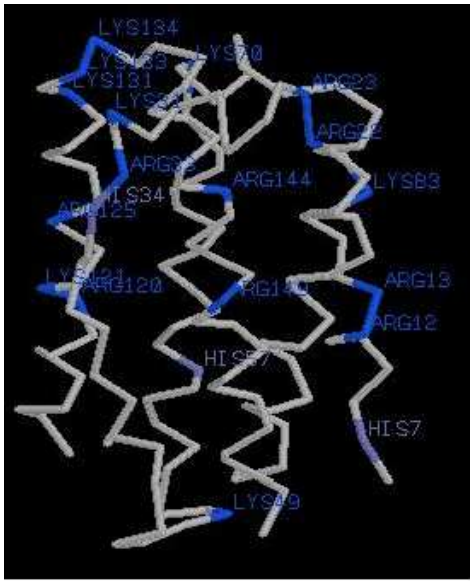


Fig. 7. Structure of an interferon, target amino acids, which can be acidified, are marked blue and violet

At least one of 3^8 derivatives will match the given receptor and the drug will have effect. Besides, appearance of such drugs can prevent a microorganism, microorganisms and viruses from adapting to the drug. For example, this may take place in case of bacteriocins modification [31]. The latter are highly specific to an exactly defined kind and sometimes strains of microorganisms. Such modification is able to widen considerably a spectrum of bacteriocins action and increase their protease stability; this will allow obtaining new peptide antibiotics on an industrial scale.

Modeling of Antagonists (completely substituted oligonucleotides)

One of perspective methods of treating a whole number of diseases is considered to be gene therapy [32]. This method is based on an intraorganism introduction of a gene being able either to induce generation of a drug in the organism (if the gene is introduced with the usage of viruses [33]) or inactivate (disable) necessary genes – the application of so-called anticomplementary oligonucleotides. The first type of genetic therapy is successfully applied in practice and quite effective in treating some kinds of leucosis, sickle-cell anemia, and a number of inherent gene diseases [34,35]. The second type of gene therapy did become so wide spread due to the fact that an inactivating anticomplementary DNA (RNA) is immediately inactivated by nucleases and does not have time to take its effect. Besides, the greater part of such anticomplementary DNA (RNA) is unable to get into a cell [36]. This makes researchers look for ways of penetrating the cell membrane by means of obtaining special plasmids or applying liposomes [37,38]. It is the second type of genetic therapy, which is the most promising for treating cancer, viral diseases, polyresistant tuberculosis and many other diseases, whose treatment involves inactivation this or that gene [39]. The binding area in such drugs was connected to one of the polynucleotide ends and represented a bis-beta-chloroethylamin derivative or other bivalent binding agent capable of inactivating a necessary gene [40]. If we obtain a completely acidified derivative on all exocyclic amino groups in the polynucleotide structure, it will be able to bind itself complementarily with its non-acidified precursor [41].

Besides, such a bond will have quite a different character – it will be an ionic bond, but not hydrogen one as between complementary chains in polynucleotides.
In places, where there are only positively charged amino groups in the precursor polynucleotide, there will be negatively charged carboxyl groups in the acidified derivative. Thus the derivative obtained will be complementary to its precursor.

This allows us to develop a number of drugs capable of not only irreversible inactivating necessary genes but of being protected from nucleases action.

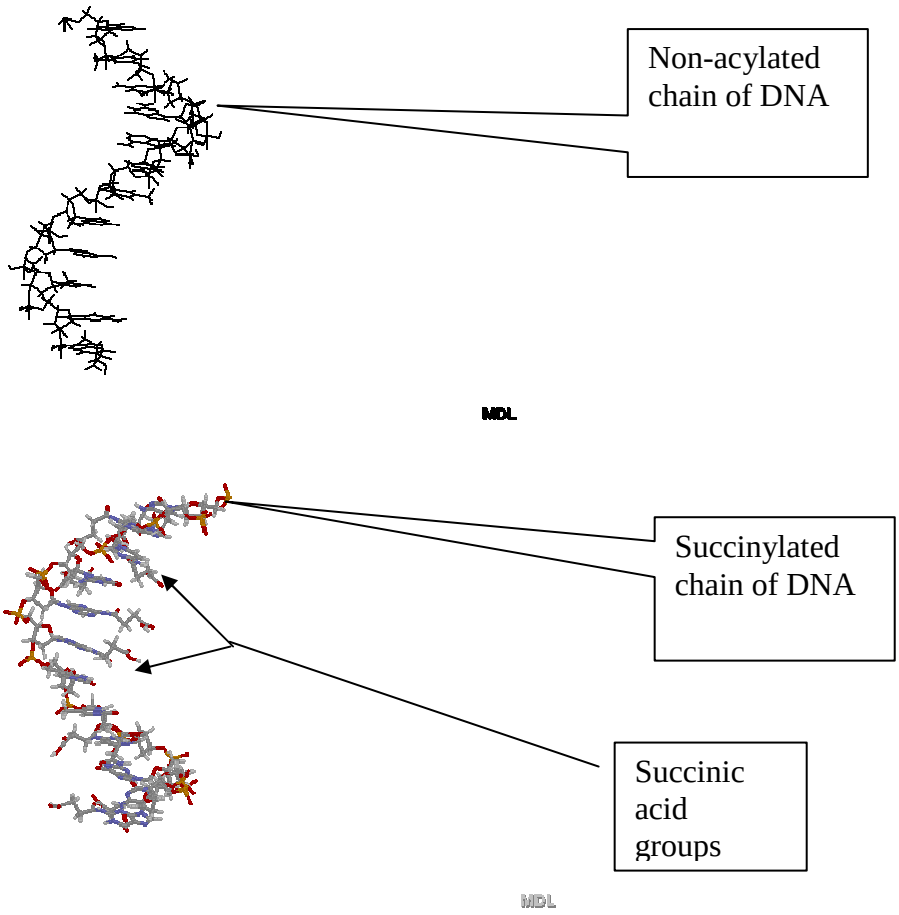


Fig. 8. Structures of non-acylated fragment of one-chain precursor DNA and its succinylated derivative complementary to its precursor

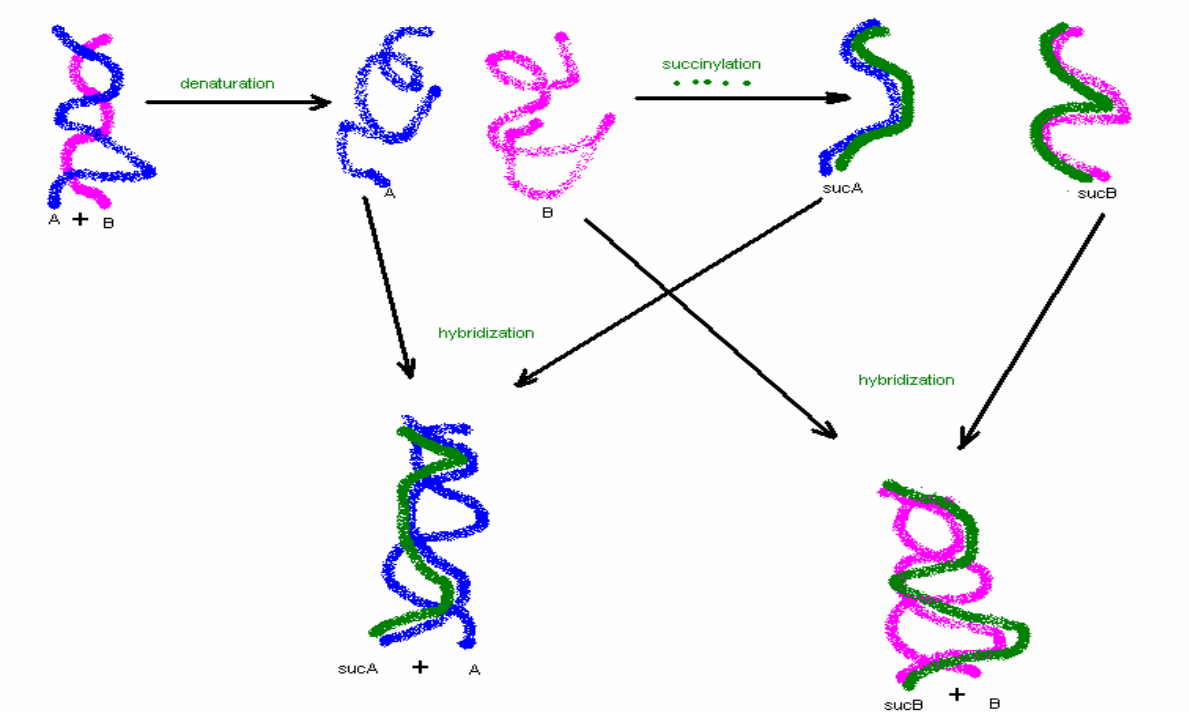


Fig.9. Process of denaturation, renaturation and hybridization between native and acylated chains of DNA.

Unlike drugs with a changed carbohydrate component (for example, to morpholine remains) [42,43,44], the double helix formed between the target polynucleotide and drug will be absolutely resistant to cell reparation systems (restrictases,

nucleases and polymerases), as in the hybridization process a principle of binding changes: a complementary ionic bond is formed, but not a hydrogen one.

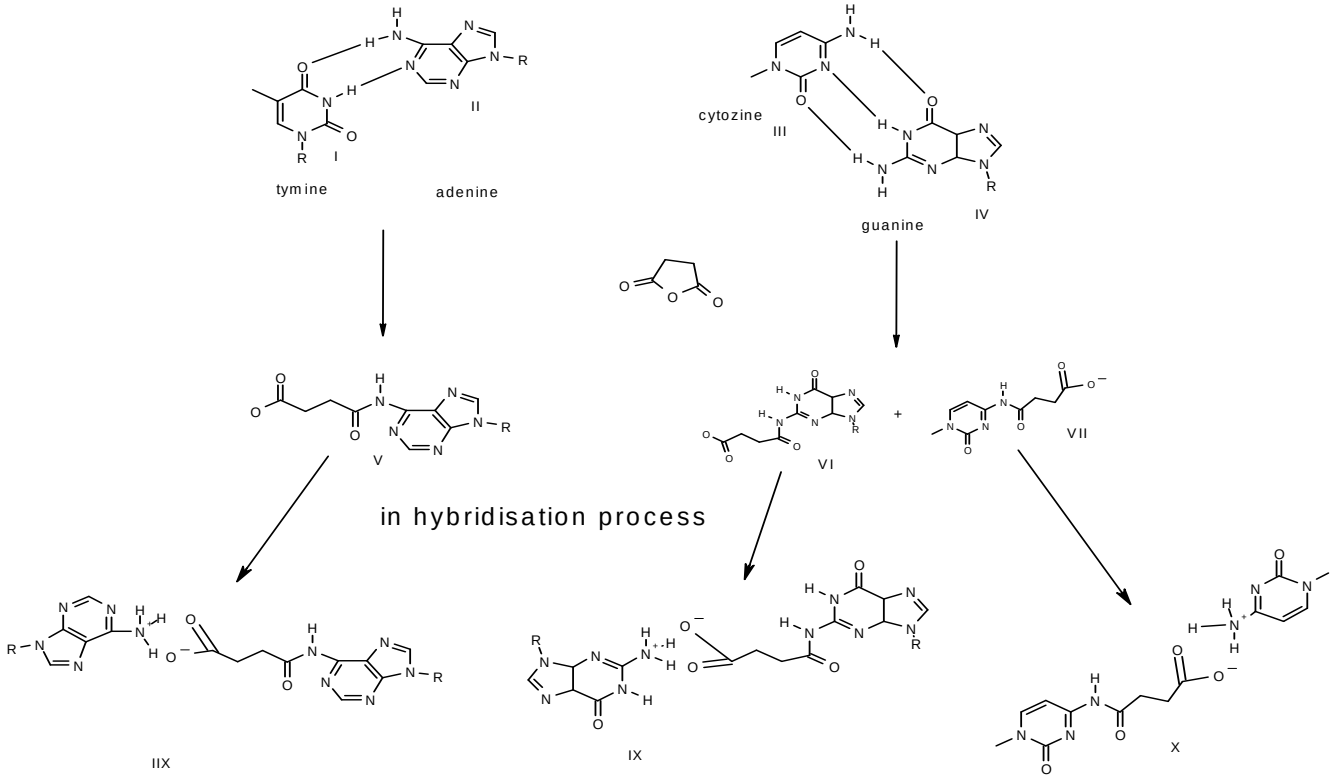


Fig.10. Hydrogen bond in the initial chain of DNA and ionic bond in a new one

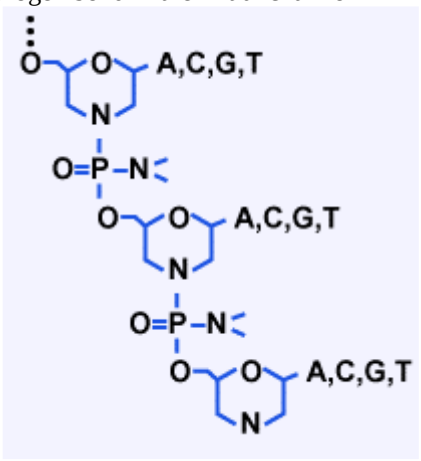


Fig.11 Polynucleotide on base of morpholine derivatives introduced by the company AVI BioPharma

Correspondingly, not a single nucleus enzyme is able to unroll such a double helix and hydrolyze or repair the blocked fragment. In this case inactivation is caused by formation of new ionic bonds between drug carboxyls and target nucleotides amino groups. Such a type of bonds remains is beyond the reach of nucleus enzymes. The main target of modeling remains target gene structure design, determination of quantity of amino groups, which can be acidified, calculation of ingredients amount. Thus, the greater part of molecular modeling tools is unnecessary, but the number of modeled and obtained drugs is unlimited.

Vaccine Development

In the modern world vaccination is one of the main methods of epidemic prevention. There are two large groups of infectious diseases: infections controlled by vaccines (their application prevents an epidemic), which are included in schemes of compulsory vaccination, and the second group of infections, whose vaccinal prevention is of low effectiveness or ineffective [45]. The first group of infections contains conservative microorganisms and viruses, whose antigenic composition is invariable and vaccine induces high levels of protective antibodies in blood. These are such infections as diphtheria, whooping cough, measles, rubella, etc. The second group of infectious diseases includes influenza, herpes infections, HIV/AIDS and some others [46,47,48]. Vaccine ineffectiveness in preventing this group of infections is caused by a whole number of factors. For example, influenza virus represents a polymorphous virus (a virus particle does not have a well-defined structure and shape) with a fragmented variable genome.

Influenza virus is very variable and capable of persisting (life-long being in a human organism) [49]. In organisms of people and animals (including birds) this virus has several phases of reproduction. During an acute reproductive phase the infected cell produces virus particles able to infect neighboring cells [50].

During the persistence (latent) phase this virus “waits through” inside the cell and loses a part of fragmented genome or catches pieces of human RNA in cytoplasm [51].

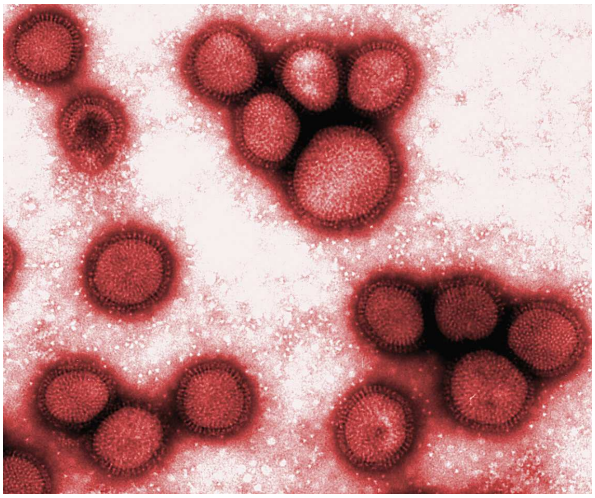


Fig.12. Electronic microphotography of influenza virus (from the site www.news.wisc.edu/ newsphotos/influenza.html). Clearly seen polymorphism of virus: virions of various sizes and shapes.

According to statistics, antigenic composition of the influenza virus changes by 5% a month [52]. Correspondingly, application of standard approaches to influenza vaccines development is not prospective. Even application of recombinant proteins and new kinds of gene vaccines does not prevent such drugs from fast obsolescence. Presence of several conservative proteins in one ampoule (for example, hemagglutinins, neuraminidases for the influenza virus) does not allow protecting the organism from the virus aggression by means of inducing specific antibodies generation. These antibodies will have a quite different monoclonal specificity, which will be necessary for such a level of virus mutation. Change of the approach to vaccine design must be accompanied by including such antigens in vaccine composition, which have not appeared as a result of virus mutation yet [53]. So called predictive inclusion is possible in two ways: in a classical one with the application of methods of antigenic drift epidemic prognosis, and by partial modification of antigens with obtaining unlimited quantity of antigen combinations in one ampoule of an antigen [54]. The first way proved only partially effective: not in a single case the antigen drift prognosis coincided with real mutational changes of influenza neuraminidase and hemagglutinin [55, 56]. If using the technology of partial modification of vaccine antigen protein component, for example, a first-type neuraminidase, in the process of vaccine preparation, one vaccine dose will contain more than a million proteins with the same primary and secondary structures, but different substitution sites and antigen profile instead of one protein with one antigen profile.

Antibodies induced by this protein will block all possible combinations of joining sites. Correspondingly, the number of induced monoclones will be one order higher, though the protein will remain the same. Any "future" epitope of the neuraminidase structure will be blocked by already synthesized antibodies.

Such an approach allows sharp reducing vaccination antigen quantity, protecting an organism from viruses with a fragmented genome and from highly variable microorganisms by means of little quantity of antibodies, but with a considerably wider spectrum of monoclonal specificity. Roughly speaking, the vaccine will even have a set of those neuraminidase antigens, which do not exist yet. At the same time, blood of animals vaccinated earlier will contain a necessary pool of antibodies to the "future" virus strain. Application of such a vaccine will allow successful protecting an organism from highly variable persisting and immunorecessive viruses and microorganisms.

Interpreter*:* Ludmila Gennadievna Starodubtseva – assistant of the department of foreign languages of M.Y. Zhukovsky National Aerospace University "KHAI"

UDC 576.851.49/ 57.083.1

NEW APPROACH TO DESIGN AND SYNTHESIS OF THERAPEUTIC AND PREVENTIVE DRUGS, TAKING INTO ACCOUNT INTERSPECIES POLYMORPHISM OF RECEPTORS (METHOD OF PRECISION PARTIAL MODIFICATION)

Martynov A.V., Smelyanskaya M.V., Peremot S.D.

In the article are presented new theory of drug's development with used of unclear and selfassembled structures – partial modified biopolymers. Also was shown perspectives in gene therapy developments and new design of actual vaccines with used principle of unclear structures .

УДК 576.851.49/ 57.083.1

НОВЫЙ ПОДХОД К ПРОЕКТИРОВАНИЮ И СИНТЕЗУ ЛЕЧЕБНЫХ И ПРОФИЛАКТИЧЕСКИХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ С УЧЕТОМ ВНУТРИВИДОВОЙ ПОЛИМОРФНОСТИ РЕЦЕПТОРОВ (МЕТОД ПРЕЦИЗИОННОЙ ЧАСТИЧНОЙ МОДИФИКАЦИИ)

Мартынов А.В., Смелянская М.В., Перемот С.Д.

В статье представлена новая теория разработки лекарственных препаратов с применением нечетких и самоорганизующихся химических структур на основе частично модифицированных биополимеров. Также показана перспективность разработки средств генной терапии и дизайна актуальных вакцин на основе принципа нечетких структур.

References:

1. Pakdaman H, Sahraian MA, Fallah A, Pakdaman R, Ghareghozli K, Ghafarpour M, Rahimian E, Shirani A. Effect of early interferon beta-1a therapy on conversion to multiple sclerosis in Iranian patients with a first demyelinating event. Acta Neurol Scand. 2007 Jun;115(6):429-31.
2. De Portu S, Menditto E, Scalone L, Bustacchini S, Cricelli C, Mantovani LG. The pharmacoeconomic impact of amlodipine use on coronary artery disease. Pharmacol Res. 2006 Aug;54(2):158-63.
3. Malcolm R, LaRowe S, Cochran K, Moak D, Herron J, Brady K, Hedden S, Woolson R, Halushka P.A controlled trial of amlodipine for cocaine dependence: a negative report. J Subst Abuse Treat. 2005 Mar;28(2):197-204.
4. Kreek MJ, Laforge KS. Stress responsivity, addiction, and a functional variant of the human mu-opioid receptor gene. Mol Interv. 2007 Apr;7(2):74-8.
5. Bigler J, Sibert JG, Poole EM, Carlson CS, Potter JD, Ulrich CM.Polymorphisms predicted to alter function in

prostaglandin E2 synthase and prostaglandin E2 receptors. Pharmacogenet Genomics. 2007 Mar;17(3):221-7.
6. Cirocco RE, Mathew JM, Burke GW 3rd, Esquenazi V, Miller J.Killer cell immunoglobulin-like receptor polymorphisms in HLA-identical kidney transplant recipients: lack of 2DL2 and 2DS2 may be associated with poor graft function. Tissue Antigens. 2007 Apr;69 Suppl 1:123-4.
7. Ahad MA, Missotten T, Abdallah A, Lympany PA, Lightman S.Polymorphisms of chemokine and chemokine receptor genes in idiopathic immune-mediated posterior segment uveitis. Mol Vis. 2007 Mar 23;13:388-96.
8. Teofoli F, Camilot M, Tato L.Lack of association between thyrotropin receptor gene polymorphisms and subclinical hypothyroidism in children. J Endocrinol Invest. 2007 Feb;30(2):163-6.
9. Jaillon P, Simon T. Genetic polymorphism of beta-adrenergic receptors and mortality in ischemic heart disease. Therapie. 2007 Jan-Feb;62(1):1-7.
10. Lundstrom K.Structural genomics and drug discovery. J Cell Mol Med. 2007 Mar-Apr;11(2):224-38.
11. Ma B, Nussinov R. Trp/Met/Phe hot spots in protein-protein interactions: potential targets in drug design. Curr Top Med Chem. 2007;7(10):1007-13. Links
12. Keskin O, Gursoy A, Ma B, Nussinov R. Towards drugs targeting multiple proteins in a systems biology approach. Curr Top Med Chem. 2007;7(10):945-53.
13. Fujii I. Directed evolution of antibody molecules in phage-displayed combinatorial libraries. Yakugaku Zasshi. 2007 Jan;127(1):91-9.
14. Galaktionov VG. Evolutionary development of the immunoglobulins super family. Izv Akad Nauk Ser Biol. 2004 Mar-Apr;(2):133-45.
15. Marchalonis JJ, Jensen I, Schluter SF. Structural, antigenic and evolutionary analyses of immunoglobulins and T cell receptors. J Mol Recognit. 2002 Sep-Oct;15(5):260-71.
16. Borisevich IV, Markin VA, Firsova IV, Evseev AA, Khamitov RA, Maksimov VA.Hemorrhagic (Marburg, Ebola, Lassa, and Bolivian) fevers: epidemiology, clinical pictures, and treatment. Vopr Virusol. 2006 Sep-Oct;51(5):8-16.
17. Martynov A.V., Smelyanskaya M.V. Antiproliferative properties of chemically modified α-2b-recombinant interferon // J. of Interf. & Cytokine res.- 2005.-Vol. 25, N 7.- P. 414-417
18. Martynov A.V., Babych E.M., Smelyanskaya M.V. Increase of vaccines adjuvanticity by succinylation of vaccine antigen// Rejuvenation Research.- Aug 2005, Vol. 8, No. 1:P.14-17
19. Chernyh V.P., Martynov A.V., Synthesis and Biological Activity of Acidified Tannins.// FAR.- 2001.- №2 (32).-P.16-19
20. Martynov A.V., Chernyh V.P. Chemical modification of high-molecular drugs – products of biotechnology as a tool of a mild influence on their pharmacological properties // Clinical Pharmacy.-2002.-V.6., № 3.-P. 3-8
21. Rodriguez-Franco MI, Fernandez-Bachiller MI, Perez C, Castro A, Martinez A Design and synthesis of N-benzylpiperidine-purine derivatives as new dual inhibitors of acetyl- and butyrylcholinesterase. Bioorg Med Chem. 2005 Dec 15;13 (24):6795-802. Epub 2005 Sep 23.
22. Hahn FE, Wisseman CL, Hopps HE. Mode of action of chloramphenicol, II. Inhibition of bacterial D-polypeptide formation by an L-stereoisomer of chloramphenicol.J Bacteriol. 1954 Jun;67(6):674-9

23 Gal J, Meyer-Lehnert S.Reversed-phase liquid chromatographic separation of enantiomeric and diastereomeric bases related to chloramphenicol and thiamphenicol.J Pharm Sci. 1988 Dec;77(12):1062-5.
24. Kireev D.B., Ros E, Bernard R, Chretien J.R., Rozhkova N.I. Computer-Aided Lead Finding and Optimization. Current Tools for Medicinal Chemistry. Wiley-VCH. 1997. P. 255.
25. H.J. Williams, G. Moyna, A.I. Scott, C.S. Swindell, L.E. Chirlian, J.M. Heerding, D.K. Williams, NMR and molecular modeling study of the conformations of Taxol 2?-acetate in chloroform and aqueous dimethyl sulfoxide solutions, J. Med. Chem. 39 (1996) 1555_ 1559.
26. R.R. Sinden, DNA Structure and Function, Academic Press, SanDiego, CA, 1994.
27. Moothoo DN, McMahon SA, Dimick SM, Toone EJ, Naismith JH.Crystallization of succinylated concanavalin A bound to a synthetic bivalent ligand and preliminary structural analysis. Acta Crystallogr D Biol Crystallogr. 1998 Sep 1;54(Pt 5):1023-5.
28. Przybylski M, Glocker MO, Nestel U, Schnaible V, Bluggel M, Diederichs K, Weckesser J, Schad M, Schmid A, Welte W, Benz R.X-ray crystallographic and mass spectrometric structure determination and functional characterization of succinylated porin from Rhodobacter capsulatus: implications for ion selectivity and single-channel conductance. Protein Sci. 1996 Aug;5(8):1477-89.
29. Ali J, Younus H.Effect of succinylation of antibodies on their conformation and interaction with the antigen. Biochemistry (Mosc). 2006 Dec;71(12):1336-40.
30. Tjiong MY, van der Vange N, ter Schegget JS, Burger MP, ten Kate FW, Out TA. Cytokines in cervicovaginal washing fluid from patients with cervical neoplasia. Cytokine. 2001 Jun 21;14(6):357-60.
31. Dykes G.A. Bacteriocins: ecological and evolutionary significanse. Trends Ecol Evol 1995; 10: 5: 186-189.
32. Hughes RM.Strategies for cancer gene therapy.J Surg Oncol. 2004 Jan;85(1):28-35.
33. Duda DG, Sunamura M, Lefter LP, Furukawa T, Yokoyama T, Yatsuoka T, Abe T, Inoue H, Motoi F, Egawa S, Matsuno S, Horii A. Restoration of SMAD4 by gene therapy reverses the invasive phenotype in pancreatic adenocarcinoma cells. Oncogene. 2003 Oct 9;22(44):6857-64.
34. Wildner O.Comparison of replication-selective, oncolytic viruses for the treatment of human cancers. Curr Opin Mol Ther. 2003 Aug;5(4):351-61.
35. Tani H, Limn CK, Yap CC, Onishi M, Nozaki M, Nishimune Y, Okahashi N, Kitagawa Y, Watanabe R, Mochizuki R, Moriishi K, Matsuura Y. In vitro and in vivo gene delivery by recombinant baculoviruses.J Virol. 2003 Sep;77(18):9799-808.
36. Wiethoff CM, Middaugh CR. Barriers to nonviral gene delivery.Pharm Sci. 2003 Feb;92(2):203-17.
37. Ignatovich IA, Dizhe EB, Pavlotskaya AV, Akifiev BN, Burov SV, Orlov SV, Perevozchikov AP. Complexes of plasmid DNA with basic domain 47-57 of the HIV-1 Tat protein are transferred to mammalian cells by endocy-

tosis-mediated pathways. J Biol Chem. 2003 Oct 24;278(43):42625-36. Epub 2003 Jul
38. Fukuhara H, Hayashi Y, Yamamoto N, Fukui T, Nishikawa M, Mitsudo K, Tohnai I, Ueda M, Mizuno M, Yoshida J Improvement of transduction efficiency of recombinant adenovirus vector conjugated with cationic liposome for human oral squamous cell carcinoma cell lines. Oral Oncol. 2003 Sep;39(6):601-9.
39. Falk T, Kilani RK, Strazdas LA, Borders RS, Steidl JV, Yool AJ, Sherman SJ. Developmental regulation of the A-type potassium-channel current in hippocampal neurons: role of the Kvbeta 1.1 subunit. Neuroscience. 2003;120(2):387-404.
40. Ardourel M, Blin M, Moret JL, Dufour T, Duc HT, Hévor T, Trojan J, Cloix JF. A New Putative Target for Antisense Gene Therapy of Glioma: Glycogen Synthase. Cancer Biol Ther. 2007 May 3;6(5)
41. Martynov A.V. Synthesis and Properties of Succinylated Polynucleotides // Pharmacy Bulletin.-2003.-№ 2 (34).-P.20-23
42. Ge Q, Pastey M, Kobasa D, Puthavathana P, Lupfer C, Bestwick RK, Iversen PL, Chen J, Stein DA.Inhibition of multiple subtypes of influenza A virus in cell cultures with morpholino oligomers. Antimicrob Agents Chemother. 2006 Nov;50(11):3724-33. Epub 2006 Sep 11.
43. Amantana A, Iversen PL.Pharmacokinetics and biodistribution of phosphorodiamidate morpholino antisense oligomers. Curr Opin Pharmacol. 2005 Oct;5(5):550-5.
44. Geller BL. Antibacterial antisense. Curr Opin Mol Ther. 2005 Apr;7(2):109-13.
45. Robbins, J. B., R. Schneerson, and S. C. Szu. 1995. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious disease by inactivating the inoculum. J. Infect. Dis. 171**:**1378-1398.
46. Del Val, M., H. J. Schlicht, H. Volkmer, M. Messerle, M. J. Reddehase, and U. H. Koszinowski. 1991. Protection against lethal cytomegalovirus infection by a recombinant vaccine containing a single nonameric T-cell epitope. J. Virol. 65:3641-3646
47. Larsen, D. L., A. Karasin, and C. W. Olsen. 2001. Immunization of pigs against influenza virus infection by DNA vaccine priming followed by killed-virus vaccine boosting. Vaccine 19**:**2842-2853
48. Cicin-Sain, L., Brune, W., Bubic, I., Jonjic, S., Koszinowski, U. H. (2003). Vaccination of Mice with Bacteria Carrying a Cloned Herpesvirus Genome Reconstituted In Vivo. J. Virol. 77: 8249-8255
49. Levin SA, Dushoff J, Plotkin JB.Evolution and persistence of influenza A and other diseases. Math Biosci. 2004 Mar-Apr;188:17-28.
50. Medvedeva MN, Petrov NA, Vasilenko SK, Simanovskaia VK, Golubev DB.The characteristics of the hemagglutinin from persistent variants of the influenza virus A/Victoria/35/72 (H3N2). Vopr Virusol. 1990 Sep-Oct;35(5):374-6.
51. Aronsson F, Robertson B, Ljunggren HG, Kristensson K. Invasion and persistence of the neuroadapted influenza virus A/WSN/33 in the mouse olfactory system. Viral Immunol. 2003;16(3):415-23.
52. Cox MM. Vaccines in development against avian influenza. Minerva Med. 2007 Apr;98(2):145-53.
53. Gronvall GK, Borio LL. Removing barriers to global pandemic influenza vaccination. Biosecur Bioterror. 2006;4(2):168-75.
54. Martynov A.V., Babych E.M., Smelyanskaya M.V. Increase of vaccines adjuvanticity by succinylation of vaccine antigen// Rejuvenation Research.- Aug 2005, Vol. 8, No. 1:P.14-17 (Poster of Confrernce)
55. Taubenberger JK, Morens DM, Fauci AS. The next influenza pandemic: can it be predicted? JAMA. 2007 May 9;297(18):2025-7.
56. Vardavas R, Breban R, Blower S. Can influenza epidemics be prevented by voluntary vaccination? PLoS Comput Biol. 2007 May 4;3(5):e85.