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1. Ponomarenko E.A., Zgoda V.G., Kopylov A.T., Poverennaya E.V., Ilgisonis E.V., Lisitsa A.V., Archakov A.I.

The Russian part of the human proteome project:first results and prospects.

The article summarizes the achievements of the pilot phase (2010-2014) of the Russian part of the international project â€œHuman Proteomeâ€• and identifies the directions for further work on the study of the human chromosome 18 proteome in the framework of the project main phase (2015-2022). The pilot phase of the project was focused on the detection of at least one protein for each chromosome 18 protein-coding gene in three types of the biological material. The application of mass spectrometric detection of proteins by the methods of multiple reactions monitoring (MRM) and gene-centric approach made it possible to detect 95% of master forms of proteins, for 60% of which the quantitative assessment of the protein content was obtained in at least one type of the biological material. The task of the main phase of the project is to measure the proteome size of healthy individuals, taking into account the modified protein forms, providing for both the bioinformatics prediction of the quantity of proteins types and the selective experimental measurement of single proteoforms. Since the ranges of protein concentrations corresponding to the normal physiological state have not been identified, the work of the main phase of the project is focused on the study of clinically healthy individuals. The absence of these data complicates significantly the interpretation of the patientsâ€™ blood proteomic profiles and prevents creating diagnostic tests. In the long term prospect, implementation of the project envisages development of a diagnostic test system based on multiple reactions monitoring (MRM) for quantitative measurement of the protein forms associated with some diseases. Development of such test systems will allow predicting the extent of risk of different diseases, diagnosing a disease at its early stage and monitoring the effectiveness of the treatment.

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2. Davydov D.R.

Molecular organization of the microsomal oxidative system: a new connotation for an old term.

The central role that cytochromes P450 play in the metabolism of drugs and other xenobiotics makes these enzymes a major subject for studies of drug disposition, adverse drug effects and drug-drug interactions. Although there has been tremendous success in delineating P450 mechanisms, the concept of the drug-metabolizing ensemble as a functionally integrated system remains undeveloped. However, eukaryotic cells typically possess a multitude of different P450 enzymes that are co-localized in the membrane of endoplasmic reticulum (ER) and interact with each other with the formation of dynamic heteromeric complexes (mixed oligomers). Appreciation of the importance of developing an integral, systems approach to the ensemble of cytochromes P450 as an integral system inspired growing interest of researchers to the molecular organization of microsomal monooxygenase, which remained in the focus of research of academician Archakov for over 40 years. Fundamental studies carried out under his guidance have an important impact on our current concepts in this area. Further exploration of the molecular organization of the system of microsomal monooxygenase as an integral multienzyme and multifunctional system will have an essential impact on our understanding of the key factors that determine the changes in human drug metabolism and other P450-related functions in development, aging, and disease, as well as under influence of drugs, food ingredients, and environmental contaminants.

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3. Shumyantseva V.V., Bulko T.V., Suprun E.V., Kuzikov A.V., Agafonova L.E., Archakov A.I.

Electrochemical methods for biomedical investigations.

In the review, authors discussed recently published experimental data concerning highly sensitive electrochemical methods and technologies for biomedical investigations in the postgenomic era. Developments in electrochemical biosensors systems for the analysis of various bio objects are also considered: cytochrome P450s, cardiac markers, bacterial cells, the analysis of proteins based on electro oxidized amino acids as a tool for analysis of conformational events. The electroanalysis of catalytic activity of cytochromes P450 allowed developing system for screening of potential substrates, inhibitors or modulators of catalytic functions of this class of hemoproteins. The highly sensitive quartz crystal microbalance (QCM) immunosensor has been developed for analysis of bio affinity interactions of antibodies with troponin I in plasma. The QCM technique allowed real-time monitoring of the kinetic differences in specific interactions and nonspecific sorption, without multiple labeling procedures and separation steps. The affinity binding process was characterized by the association (ka) and the dissociation (kd) kinetic constants and the equilibrium association (K) constant, calculated using experimental data. Based on the electroactivity of bacterial cells, the electrochemical system for determination of sensitivity of the microbial cells to antibiotics cefepime, ampicillin, amikacin, and erythromycin was proposed. It was shown that the minimally detectable cell number corresponds to 106 CFU per electrode. The electrochemical method allows estimating the degree of E.coli JM109 cells resistance to antibiotics within 2-5 h. Electrosynthesis of polymeric analogs of antibodies for myoglobin (molecularly imprinted polymer, MIP) on the surface of graphite screen-printed electrodes as sensor elements with o- phenylenediamine as the functional monomer was developed. Molecularly imprinted polymers demonstrate selective complementary binding of a template protein molecule (myoglobin) by the "key - lock" principle.

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4. Radko S.P., Khmeleva S.A., Suprun E.V., Kozin S.A., Bodoev N.V., Makarov A.A., Archakov A.I., Shumyantseva V.V.

Physico-chemical methods for studing beta-amyloid aggregation.

Alzheimer's disease is the most prevalent neurodegenerative pathology. According to the amyloid cascade hypothesis, a key event of the Alzheimer's

disease pathogenesis is a transition of the b-amyloid peptide (Ð•b) from the monomeric form to the aggregated state. The mechanism of Ð•b aggregation is intensively studied in vitro, by means of synthetic peptides and various physico-chemical methods allowing evaluation of size, molecular structure, and morphology of the formed aggregates. The paper reviews both the well-known and recently introduced physico-chemical methods for analysis of Ð•b aggregation, including microscopÑƒ, optical and fluorescent methods, method of electron paramagnetic resonance, electrochemical and electrophoretic methods, gel-filtration, and mass spectrometric methods. Merits and drawbacks of the methods are discussed. The unique possibility to simultaneously observe Ð•b monomers as well oligomers and large aggregates by means of atomic force microscopy or fluorescence correlation spectroscopy is emphasized. The high detection sensitivity of the latter method, monitoring the aggregation process in Ð•b solutions at low peptide concentrations is underlined. Among mass spectrometric methods, the ion mobility mass spectrometry is marked out as a method enabling to obtain information about both the spectrum of Ð•b oligomers and their structure. It is pointed out that the use of several methods giving the complementary data about Ð•b aggregates is the best experimental approach to studying the process of b-amyloid peptide aggregation in vitro.
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5. Medvedeva N.V., Prosorovskiy V.N., Ignatov D.V., Druzilovskaya O.S., Kudinov V.A., Kasatkina E.O., Tikhonova E.G., Ipatova O.M.

Pharmacological agents and transport nanosystems based on plant phospholipids.

A new generation of plant phosphatidylcholine (PC)-based pharmacological agents has been developed under academician A.I. Archakov leadership at the Institute of Biomedical Chemistry (IBMC). For their production a unique technology allowing to obtain dry lyophilized phospholipid nanoparticles of 30 nm was elaborated. The successful practical application of PC nanoparticles as a drug agent may be illustrated by Phosphogliv (oral and injection formulations). Being developed at IBMC for the treatment of liver diseases, including viral hepatitis, Phosphogliv (currently marketed by the â€œPharmstandardâ€• company) is approved for clinical application in 2000, and is widely used in medical practice. Based on the developed and scaled in IBMC technology of prerparation of ultra small size phospholipid nanoparticles without the use of detergents/surfactants and stabilizers another drug preparation, Phospholipovit, exhibiting pronounced hypolipidemic properties has been obtained. Recently completed preclinical studies have shown that PC nanoparticles of 20-30 nm activate reverse cholesterol transport (RCT) and in this context it is more active than well known foreign preparation Essentiale. Phospholipovit is now at the stage of clinical trials (phase 1 completed). PC was also used as a basis for the development of a transport nanosystem with a particles size of 20-25 nm in diameter and incorporation of various drug substances from various therapeutic groups. Using several drugs substances as an example, increased bioavailability and specific activity were demonstrated for the formulations equipped with such transport nanosystem. Formulations equipped with the transport nanosystems have been developed for such pharmacological agents as doxorubicin, rifampin, budesonide, chlorin E6, prednisone, and others.
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6. Ivanov A.S., Medvedev A.E.

Optical surface plasmon resonance biosensors in molecular fishing.

An optical biosensor employing surface plasmon resonance is a highly efficient instrument applicable for direct real time registration of molecular interactions without additional use of any labels or coupled processes. As an independent approach it is especially effective in analysis of various ligand receptor interactions. SPR-biosensors are used for validation of studies on intermolecular interactions in complex biological systems (affinity profiling of various groups of proteins, etc.). Recently, potential application of the SPR-biosensor for molecular fishing (direct affinity binding of target molecules from complex biological mixtures on the optical biosensor surface followed by their elution for identification by LC-MS/MS) has been demonstrated. Using SPR-biosensors in such studies it is possible to solve the following tasks: (a) SPR-based selection of immobilization conditions required for the most effective affinity separation of a particular biological sample; (b) SPR-based molecular fishing for subsequent protein identification by mass spectrometry; (c) SPR-based validation of the interaction of identified proteins with immobilized ligand. This review considers practical application of the SPR technology in the context of recent studies performed in the Institute of Biomedical Chemistry on molecular fishing of real biological objects.
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7. Pleshakova T.O., Shumov I.D., Ivanov Yu.D., Malsagova K.A., Kaysheva A.L., Archakov A.I.

AFM-based technologies as the way towards the reverse Avogadro number.

Achievement of the concentration detection limit for proteins at the level of the reverse Avogadro number determines the modern development of proteomics. In this review, the possibility of approximating the reverse Avogadro number by using nanotechnological methods (AFM-based fishing with mechanical and electrical stimulation, nanowire detectors, and other methods) are discussed. The ability of AFM to detect, count, visualize and characterize physico-chemical properties of proteins at concentrations up to 10-17-10-18 M is demonstrated. The combination of AFM-fishing with mass-spectrometry allows the identification of proteins not only in pure solutions, but also in multi-component biological fluids (serum). The possibilities to improve the biospecific fishing efficiency by use of SOMAmers in both AFM and nanowire systems are discussed. The paper also provides criteria for evaluation of the sensitivity of fishing-based detection systems. The fishing efficiency depending on the detection system parameters is estimated. The practical implementation of protein fishing depending on the ratio of the sample solution volume and the surface of the detection system is discussed. The advantages and disadvantages of today's promising nanotechnological protein detection methods implemented on the basis of these schemes.
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8. Kolesanova E.F., Sobolev B.N., Moysa A.A., Egorova E.A., Archakov A.I.

Way to the peptide vaccine against hepatitis C.

In order to surpass the problem of genetic variability of hepatitis C virus envelope proteins during vaccine development, we used the so-called â€œreverse vaccinologyâ€•approach â€“ â€œfrom genome to vaccineâ€•. Database of HCV protein sequences was designed, viral genome analysis was performed, and several highly conserved sites were revealed in HCV envelope proteins in the framework of this approach. These sites demonstrated low antigenic activity in full-size proteins and HCV virions: antibodies against these sites were not found in all hepatitis C patients.

However, two sites, which contained a wide set of potential T-helper epitope motifs, were revealed among these highly conserved ones. We constructed and prepared by solid-phase peptide synthesis several artificial peptide constructs composed of two linker-connected highly conserved HCV envelope E2 protein sites; one of these sites contained a set of T-helper epitope motifs. Experiments on laboratory animals demonstrated that the developed peptide constructs manifested immunogenicity compared with one of protein molecules and were able to raise antibodies, which specifically bound HCV envelope proteins. We succeeded in obtaining antibodies reactive with HCV from hepatitis C patient plasma upon the immunization with some constructs. An original preparation of a peptide vaccine against hepatitis C is under development on the basis of these peptide constructs.
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9. Veselovsky A.V., Ivanov A.S., Medvedev A.E.

Computer modelling of monoaminoxidases.

The article summarized results of studies on active site structures of monoamine oxidases (MAO) performed in the Institute of Biomedical Chemistry (Russia) by computer modelling approaches. MAO, catalyzing the reaction of oxidative deamination of major neurotransmitter monoamines, exists in two highly homologous forms, MAO A and MAO B, distinguished by substrate specificity and inhibitor selectivity. The development of approaches for active site modelling of these enzymes (with unknown three-dimensional structures) started from analysis of relationship between the geometrical sizes of rigid indole and isatin derivatives and their inhibitory activity. These studies resulted in molding of the active site structures of MAO A and MAO B. These molds reflect the sizes and shapes of active sites of these enzymes. These mold models have been used for virtual screening of molecular databases for new inhibitors. The models obtained at different stages ofMAO investigations have been compared with recently appeared three-dimensional structures of MAO A and MAO B.
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10. Miroshnichenko Iu.V., Petushkova N.A., Moskaleva N.E., Teryaeva N.B., Zgoda V.G., Ilgisonis E.V., Belyaev A.Yu.

The possibility of using PlasmaDeepDiveâ„¢ MRM panel in clinical diagnostics.

Concentrations of 46 proteins have been determined in human blood plasma using PlasmaDeepDiveâ„¢ MRM Panel ("Biognosys AG", Switzerland). 18 of them were included into the group of proteins with higher concentrations, also identified by the shotgun proteomic analysis. Based on literature data it is concluded that the PlasmaDeepDiveâ„¢ MRM Panel is applicable for studies of human plasma samples for potential biomarkers of various nervous system disorders.
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11. Naryzhny S.N., Zgoda V.G., Maynskova M.A., Ronzhina N.L., Belyakova N.V., Legina O.K., Archakov A.I.

Experimental estimation of proteome size for cells and human plasma.

Huge range of concentrations of different protein and insufficient sensitivity of methods for detection of proteins at a single molecule level does not yet allow obtaining the whole image of human proteome. In our investigations, we tried to evaluate the size of different proteomes (cells and plasma). The approach used is based on detection of protein spots in 2-DE after staining by protein dyes with different sensitivities. The function representing the dependence of the number of protein spots on sensitivity of protein dyes was generated. Next, by extrapolation of this function curve to theoretical point of the maximum sensitivity (detection of a single smallest polypeptide) it was calculated that a single human cell (HepG2) may contain minimum 70000 proteoforms, and plasma â€“ 1.5 mln. Utilization of this approach to other, smaller proteomes showed the competency of this extrapolation. For instance, the size of mycoplasma (Acholeplasma laidlawii) was estimated in 1100 proteoforms, yeast (Saccharomyces cerevisiae) - 40000, E. coli â€“ 6200, P. furiosus â€“ 3400. In hepatocytes, the amount of proteoforms was the same as in HepG2 â€“ 70000. Significance of obtained data is in possibilities to estimating the proteome organization and planning next steps in its study.
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12. Lagunin A.A., Druzhilovsky D.S., Rudik A.V., Filimonov D.A., Gawande D., Suresh K., Goel R., Poroikov V.V.

Computer evaluation of hidden potential of phytochemicals of medicinal plants of the traditional indian ayurvedic medicine.

Applicability of our computer programs PASS and PharmaExpert to prediction of biological activity spectra of rather complex and structurally diverse phytocomponents of medicinal plants, both separately and in combinations has been evaluated. The web-resource on phytochemicals of 50 medicinal plants used in Ayurveda was created for the study of hidden therapeutic potential of Traditional Indian Medicine (TIM) (http://ayurveda.pharmaexpert.ru). It contains information on 50 medicinal plants, their using in TIM and their pharmacology activities, also as 1906 phytocomponents. PASS training set was updated by addition of information about 946 natural compounds; then the training procedure and validation were performed, to estimate the quality of PASS prediction. It was shown that the difference between the average accuracy of prediction obtained in leave-5%-out cross-validation (94,467%) and in leave-one-out cross-validation (94,605%) is very small. These results showed high predictive ability of the program. Results of biological activity spectra prediction for all phytocomponents included in our database are in good correspondence with the experimental data. Additional kinds of biological activity predicted with high probability provide the information about most promising directions of further studies. The analysis of prediction results of sets of phytocomponents in each of 50 medicinal plants was made by PharmaExpert software. Based on this analysis, we found that the combination of phytocomponents from Passiflora incarnata may exhibit nootropic, anticonvulsant and antidepressant effects. Experiments carried out in mice models confirmed the predicted effects of Passiflora incarnata extracts.
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