

4th International Conference
“Genomics, Proteomics, Bioinformatics
and Nanobiotechnologies for Medicine”

Moscow - Nizhny Novgorod - Moscow
Russia

June 1-7, 2008



Abstract Book

<http://www.ibmc.msk.ru/gpbnm2008>

Major Sponsors:



HT-Lab AG



InterLab

<http://www.interlab.ru/>



Pharmstandart

<http://www.pharmstd.ru/>

Sponsors:



Applied Biosystems

<http://www.appliedbiosystems.com>



Beckman Coulter

<http://www.beckmancoulter.com/>



BIO-RAD Laboratories

<http://www.biorad.ru>



NT-MDT

<http://www.ntmdt.ru/>



HVD Biotech

<http://www.hvd.ru/>



Acrus

<http://www.acrus.ru>

**4th International Conference
“Genomics, Proteomics, Bioinformatics and
Nanobiotechnologies for Medicine”
(GPBNM-2008)**

**Moscow - Nizhny Novgorod - Moscow
June 1-7, 2008**

Organizers:

- **International Union of Biochemistry and Molecular Biology (IUBMB)**
- **International Human Proteome Organization (HUPO)**
- **International Science and Technology Center (ISTC)**
- **Federal Agency on Science and Innovation**
- **The Ministry of Health and Social Development of Russian Federation**
- **Russian Academy of Medical Sciences (RAMS)**
- **Russian Foundation for Basic Research (RFBR)**
- **Moscow Committee on Science and Technologies (MCST)**
- **Moscow Department of Science and Industrial Policy**
- **Institute of Biomedical Chemistry RAMS**
- **Noncommercial Partnership “Center of Proteomic Researches”**

Organizing Committee:

Davydov M.I.	Co-Chairman
Starodubov V.I.	Co-Chairman
Archakov A.I.	Vice-Chairman
Pokrovsky V.I.	Vice-Chairman
Sagdeev R.Z.	Vice-Chairman

Balashov E.B.	Gremyakova T.A.	Lisitsa A.V.	Shipulin G.A.
Bilenkina I.P.	Egorov A.M.	Nikolayev E.N.	Tsyganov D.I.
Bykov Valery A.	Ivanov Yu.D.	Podoplelov A.V.	Tuteljan V.A.
Bykov Victor A.	Ipatova O.M.	Rototaev D.A.	Vedenin A.N.
Govorun V.M.	Kolesnikov S.I.	Shevchenko V.E.	Voitkevich N.D.

International Scientific Committee:

Bairoch A.	Hanash S.	Kovalchuk M.V.	Severin E.S.
Bernhardt R.	Hochstrasser D.	Makarov A.A.	Sligar S.
Brechot C.	Humphery-Smith I.	Omenn G.	Sverdlov E.D.
Debabov V.G.	Ingelman-Sundberg M.	Paik Y.-K.	Tkachuk V.A.
Georgiev G.P.	Ivanov V.T.	Petrov R.V.	Vlasov V.V.
Grigoriev A.I.	Kel A.	Pompon D.	Zaridze D.G.
Guengerich P.	Khaitov R.M.	Skrjabin K.G.	

Local Committee:

Poroikov V.V.	Chairman
Guseva M.K.	Vice-Chairman
Ivanov A.S.	Vice-Chairman
Karpova E.A.	Secretary

Berestova O.N.	Fedoronchuk T.V.	Rozhnova E.O.	Tarasova E.A.
Beyer E.M.	Ponomarenko E.A.	Shadrina T.N.	

N	PROGRAM	
---	---------	--

1 June 2008		Time
Arrival to Moscow and Transportation to Northern River Port		
Accommodation on the ship "Alexander Suvorov"		16.00
THE OPENING CEREMONY		19.00
<i>Welcoming words:</i>		
1	Davydov M.I. President of Russian Academy of Medical Sciences	
2	Mazurenko S.N. Head of Federal Agency on Science and Innovation	
3	Panteleev E.A. Head of Science and Industry Department of Moscow Government	
4	Archakov A.I. Director of Institute of Biomedical Chemistry, Rus.Acad.Med.Sci.	
Welcome Party		19.30
Departure from Moscow		21.30

2 June 2008		Time
Breakfast		8.00 - 9.00
SESSION 1. PROTEOMICS (Part 1)		9.00 -11.00
<i>Chairpersons: Laura Beretta, David Zaridze</i>		
1	Laura Beretta, Fred Hutchinson Cancer Research Centre Seattle, Washington USA WHAT CAN PROTEOMICS DELIVER TO MOLECULAR MEDICINE: EXAMPLE OF HEPATITIS C VIRUS AND ASSOCIATED LIVER DISEASES	9.00 -9.30
2	David Zaridze, Institute of Carcinogenesis, Russian Oncological Scientific Center, Moscow, Russia Title of the talk is expected	9.30-10.00
3	Young-Ki Paik, Yonsei Proteome Research Center, Seoul, Korea A COMPREHENSIVE STRATEGY FOR THE DISCOVERY AND VALIDATION OF HCC BIOMARKER	10.00-10.30
4	Andrey Lisitsa, Institute of Biomedical Chemistry, RAMS, Moscow, Russia PROTEOMIC MAPPING OF CYTOCHROMES P450 BY JOINING MASS-SPECTRAL INFORMATION FROM ADJACENT SLICES OF SDS-PAGE	10.30-11.00
Coffee Break		11.00 - 11.15
SESSION 1. PROTEOMICS (Part 2)		11.15 - 13.00
<i>Chairpersons: Young-Ki Paik, Rita Bernhardt</i>		
1	Alexis Ivanov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia INTERACTION OF OLIGOMERIC ENZYMES SUBUNITS IN SILICO AND IN VITRO	11.15 - 11.45
2	Rita Bernhardt, University of Saarland, Saarbrücken, Germany STEROID HORMONE ACTION ON THE PROTEOME STUDIED USING FISSION YEAST AS A MODEL	11.45 - 12.15
3	Sergei Moshkovskiy, Institute of Biomedical Chemistry, RAMS, Moscow, Russia PLASMA PROTEOME PROFILING BY MALDI-TOF-MASS-SPECTROMETRY FOR CANCER DIAGNOSTICS	12.15 - 12.45
4	Jan Zivny, Department of Pathophysiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic PROTEOMICS REVEALS MOLECULAR TARGETS FOR ELIMINATION OF THERAPY-RESISTANT LEUKEMIA CELLS.	12.45 -12.55
Lunch		13.00 - 14.00
SESSION 1. PROTEOMICS (Part 3)		14.30 - 16.30

Chairpersons: Valery Shevchenko, Suresh Jivan Gadher		
1	Suresh Gadher, Bekman Coulter International S.A., Nyon, Switzerland PROTEIN FRACTIONATION AND RELATIVE QUANTITATION USING PF 2D AND ITRAQ FOR BIOMARKER QUEST	14.30 - 15.00
2	Valery Shevchenko, Institute of Carcinogenesis, Russian Oncological Scientific Center, Moscow, Russia Title of the talk is expected	15.00 - 15.30
3	Evgeny Nikolaev, Institute of Biomedical Chemistry, RAMS, Moscow, Russia POTENTIALS OF MASS-SPECTROMETRY IN PROTEOMICS	15.30 - 16.00
4	María Pajares, Instituto de Investigaciones Biomédicas Alberto Sols Madrid, Spain. NEW APPROACHES FOR THE IDENTIFICATION OF PROTEINS INTERACTING WITH ENZYMES OF HEPATIC METHIONINE METABOLISM	16.00 - 16.10
5	Nikolay Bodoev, Institute of Biomedical Chemistry, RAMS, Moscow, Russian APTAMER MULTIMERIC CONSTRUCTS – A NEW ROAD IN DEVELOPING SYNTHETIC AFFINITY REAGENTS FOR CHIP-BASED PROTEOMICS?	16.10- 16.20
Coffee Break		16.20 - 16.40
SESSION 2. GENOMICS (Part 1)		16.40 - 19.30
Chairpersons: Steve Kelly, Vadim Govorun		
1	Steve Kelly, Institute of Life Science and School of Medicine, Swansea University, Swansea, UK FUNCTIONAL GENOMIC STUDIES IN MICROBIAL CYTOCHROMES P450 (CYP)	16.40-17.10
2	Vadim Govorun, Institute of Physico-Chemical Medicine, Moscow, Russia PROTEOGENOMIC PROFILING OF ACHOLEPLASMA LAIDLAVII	17.10- 17.40
3	Dolph Hatfield, National Cancer Institute, NIH, Bethesda, USA HOW SELENIUM HAS CHANGED OUR UNDERSTANDING OF THE GENETIC CODE	17.40- 18.10
4	Vadim Gladyshev Redox Biology Center and Department of Biochemistry, University of Nebraska, Lincoln, USA HIGH-THROUGHPUT IDENTIFICATION OF CATALYTIC REDOX-ACTIVE CYSTEINE RESIDUES AND SELENOPROTEIN GENES	18.10- 18.30
5	Tatyana Gremyakova, ISTC, Moscow, Russia DRUG DESIGN AND DEVELOPMENT ISTC TARGETED INITIATIVE	18.30- 18.50
6	Elena Iliina, Research Institute of Physico-Chemical Medicine, Moscow, Russia DIAGNOSTIC PYRAMID FOR BACTERIAL IDENTIFICATION AND TYPING	18.50- 19.10
7	Edward Generozov, Institute of Physico-Chemical Medicine, Moscow, Russia MALDI-TOF MASS-SPECTROMETRY AS A TOOL FOR ANALYSIS OF GENOMIC SEQUENCE POLYMORPHISMS. HIGH-THROUGHPUT SCREENING OF GENETIC ALTERATIONS IN RUSSIAN COLORECTAL CANCER PATIENTS	19.10- 19.20
8	Alexei Lupatov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia FULL GENOME TRANSCRIPTOM ANALYSIS OF T-REGULATORY CELLS ISOLATED FROM CANCER PATIENTS	19.20- 19.30
9	Olga Voronko, Institute of Biomedical Chemistry, RAMS, Moscow, Russia ANALYSIS OF GENETIC SUSCEPTIBILITY FOR BRONCHIAL ASTHMA IN RUSSIANS	19.30-19.40
Dinner		19.40- 20.30
Uglich city tour		20.30 -22.00
Departure from Uglich		22.30

	3 June 2008	
	Breakfast	9.00 - 10.00
	SESSION 3. BIOINFORMATICS (Part 1)	10.00 - 11.00
	<i>Chairpersons: Herbert Thiele, Alexander Veselovsky</i>	
1	Herbert Thiele, Bruker Daltonik, Bremen, Germany BIOINFORMATICS STRATEGIES FOR DIAGNOSIS AND BIOMARKER DISCOVERY IN MALDI-TOF MS PROFILES	9.00- 9.30
2	Nikolaus Rajewsky, Max Delbrück Center for Molecular Medicine, Berlin-Buch, Germany IDENTIFYING MICRIRNAS AND THEIR TARGETS	9.30- 10.00
3	Eugene Korotkov, Bioengineering Centre, RAS, Moscow, Russia CLASSIFICATION OF TRIPLET PERIODICITY IN DNA SEQUENCES OF KNOWN GENES TAKEN FROM KEGG DATABANK	10.00- 10.30
4	Alexander Veselovsky, Institute of Biomedical Chemistry, RAMS, Moscow, Russia VIRTUAL SCREENING OF INHIBITORS OF HIV PROTEASE DIMERIZATION USING MM-PBSA METHOD	10.30-10.45
	Coffee Break	11.00 - 11.30
	SESSION 3. BIOINFORMATICS (Part 2)	11.30 - 13.00
	<i>Chairpersons: Jiri Petrak, Alexis Ivanov</i>	
1	Jiri Petrak, Institute of Hematology and Blood Transfusion, Prague, Czech Republic ENOLASE AND HEAT SHOCK PROTEINS AGAIN? THE FIRST PROTEOMIC HIT PARADE OF REPEATEDLY IDENTIFIED DIFFERENTIALLY EXPRESSED PROTEINS	11.30- 12.00
2	Adel Golovin, The European Bioinformatics Institute, Cambridge, UK PDB LIGANDS, SITES AND MOTIFS DATABASE WITH THE SEARCH ENGINE	12.00- 12.20
3	Elena Ponomarenko, Institute of Biomedical Chemistry, RAMS, Moscow, Russia TEXTOMICS TOOLS FOR AUTOMATICALLY UPDATE THE HIT-PARADE OF REPEATEDLY IDENTIFIED PROTEINS	12.20- 12.30
4	Olga Koborova, Institute of Biomedical Chemistry, RAMS, Moscow, Russia METHOD OF PERSPECTIVE TARGET ANALYSIS FOR BREAST CANCER THERAPY	12.30- 12.40
5	Mikhail Pyatnitskiy, Institute of Biomedical Chemistry, RAMS, Moscow, Russia PREDICTION OF FUNCTIONALLY RELATED PROTEINS: PHYLOGENETIC PROFILES AND CLUSTER ANALYSIS	12.40- 12.50
	Lunch	14.00 - 15.00
	SESSION 3. BIOINFORMATICS (Part 3)	15.0- 17.00
	<i>Chairpersons: Marc Nicklaus, Vladimir Poroikov</i>	
1	Marc Nicklaus, National Cancer Institute, NIH, Frederick, USA LARGE DATABASE RESOURCES AND WEB TOOL FOR DRUG DEVELOPMENT	15.00- 15.30
2	Vladimir Poroikov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia BIO- AND CHEMINFORMATICS APPROACHES TO DISCOVERY OF NEW ANTINEOPLASTIC TARGETS AND LIGANDS	15.30- 16.00
3	Jürgen Pleiss, Institute of Technical Biochemistry, Stuttgart, Germany SHAPE, FLEXIBILITY, REACTIVITY: A MOLECULAR MODEL OF REGIOSELECTIVITY OF CYTOCHROME P450 MONOOXYGENASES	16.00- 16.30
4	Dmitry Filimonov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia RECOGNITION OF PROTEIN FUNCTION USING THE LOCAL SIMILARITY	16.30- 16.45

5	Yuriy Koptsov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia LABORATORY INFORMATION MANAGEMENT SYSTEM "PROTEY"	16.45- 16.55
	Plyos city tour	18.00 - 21.00
	Dinner	21.00 - 22.00
	Departure from Plyos	21.00

	4 June 2008	
	Breakfast	8.00 - 9.00
	Nizhny Novgorod city tour	9.00 - 19.00
	Dinner	19.30 - 20.30
	Departure from Galanino	19.00

	5 June 2008	
	Breakfast	8.00 - 9.00
	SESSION 4. NANOBIOLOGICALS AND NANOMEDICINE (Part 1)	9.00 - 11.00
	Chairpersons: Manfred Radmacher, Viktor Bykov	
1	Manfred Radmacher, Institute of Biophysics, University of Bremen, Germany INVESTIGATION OF CELLULAR MECHANICS BY AFM	9.00 - 9.30
2	Viktor Bykov Joint-Stock Company "NT-MDT", Russia NANOTECHNOLOGY TOOL FOR SOLVING IDENTIFICATION PROBLEMS AND NANOMANIPULATIONS AT SUB-CELLULAR LEVEL	9.30- 10.00
3	Yuri Ivanov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia NANOCHIPS FOR MEDICAL DIAGNOSTICS	10.00- 10.30
4	Viktoria Shumiantseva, Institute of Biomedical Chemistry, RAMS, Moscow, Russia NANOBIOELECTROCHEMICAL METHODS FOR THE INVESTIGATION OF BIOAFFINITY	10.30- 11.00
	Coffee Break	11.00 - 11.10
	SESSION 4. NANOBIOLOGICALS AND NANOMEDICINE (Part 2)	11.10 - 13.00
	Chairpersons: Alexander Archakov, Evgeniy Severin	
1	Alexander Archakov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia ANALYTICAL NANOTECHNOLOGY IN PROTEOMICS ON THE WAY TO REVERSE AVOGADRO NUMBER	11.10- 11.40
2	Olga Ipatova, Institute of Biomedical Chemistry, RAMS, Moscow, Russia NANOPHARMACEUTICALS: TODAY AND TOMORROW	11.40- 12.10
3	Elena Kiseleva, Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia MEMBRANE NANO-TUBES IN LIVING CELL AND MECHANISM OF THEIR FORMATION	12.10- 12.20
4	Maria Lanio, Universidad de la Habana, Cuba NEW STRATEGY FOR THE DESIGN OF CYTOSOLIC DELIVERY SYSTEMS BASED ON STICHOLOSINS, PORE-FORMING PROTEINS, ENCAPSULATED INTO LIPOSOMES	12.20- 12.30

5	Hana Kovarova, Institute of Animal Physiology and Genetics AS CR, Libechov, Czech Republic QUEST FOR UNIQUE BIOMARKERS IN HUMAN FOLLICULAR FLUID	12.30- 12.45
	Lunch	13.00 - 14.00
	Yaroslavl city tour	15.00 - 19.00
	Dinner	19.30 - 20.30
	Departure from Yaroslavl	19.00

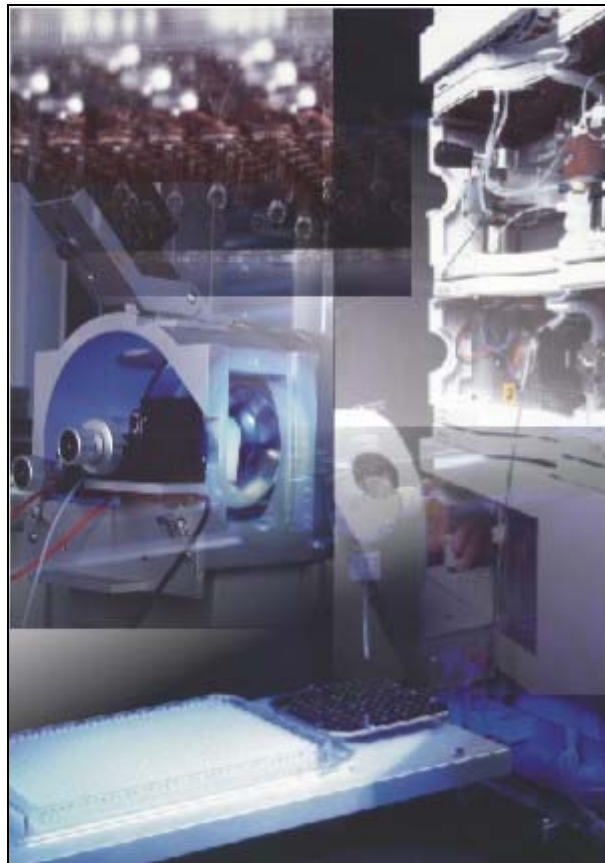
	6 June 2008	
	Breakfast	8.00 - 9.00
	SESSION 5. SYSTEMS BIOLOGY (Part 1)	9.00 - 10.40
	Chairpersons: Alexander Kel, Viktor Zgoda	
1	Alexander Kel, SVP R&D Biobase GmbH, Wolfenbuettel, Germany NETWORK BIOLOGY OF CELL DIFFERENTIATION	9.00- 9.30
2	Viktor Zgoda, Institute of Biomedical Chemistry, RAMS, Moscow, Russia GENE EXPRESSION AND PROTEOMIC PROFILING OF INDUCED HEPATOTOXICITY IN MICE	9.30- 10.00
3	Rudolf Grimm, Agilent Technologies, Santa Clara, USA LATEST ADVANCEMENTS IN HPLC-CHIP/MS WITH APPLICATIONS TO PROTEOMICS AND GLYCOMICS RESEARCH	10.00- 10.30
	Coffee Break	10.40 - 11.00
	SESSION 5. SYSTEMS BIOLOGY (Part 2)	11.00 - 13.30
	Chairpersons: Rudolf Grimm, Konstantin Yarygin	
1	David Balshaw, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, USA BIOLOGICAL ROBUSTNESS AND THE ENVIRONMENTAL ORIGINS OF 'COMPLEX' HUMAN DISEASES	11.00- 11.30
2	Konstantin Yarygin, Institute of Biomedical Chemistry, RAMS, Moscow, Russia METHODS OF THE PREPARATION OF CELLS FOR THE SYSTEMS BIOLOGY STUDIES	11.30- 12.00
3	Murat Gainullin, Nizhny Novgorod State Medical Academy, Russia A SYSTEMS BIOLOGY STRATEGY FOR THE STUDIES OF THE UBIQUITIN SYSTEM	12.00- 12.30
	SESSION 6. SYNTHETIC BIOLOGY	12.30- 13.40
	Chairpersons: Clyde Hutchison III, Ekaterina Kolesanova	
1	Clyde Hutchison III, The J. Craig Venter Institute, San-Diego, USA THE QUEST FOR A MINIMAL CELL: CONSTRUCTING A SYNTHETIC MYCOPLASMA GENITALIUM GENOME	12.30- 13.00
2	Ekaterina Kolesanova, Institute of Biomedical Chemistry, RAMS, Moscow, Russia SYNTHETIC PEPTIDE ARRAYS IN SEARCH FOR DIAGNOSTIC ANTIBODIES AND VACCINE DEVELOPMENT	13.00- 13.30
3	Carlos Alvarez, Center for Protein Studies, University of Havana, Cuba STICHOLYSIN II STRUCTURE AND FUNCTION CAN BE MODELLED BY SYNTHETIC PEPTIDES REPRODUCING THE N-TERMINUS OF THIS PORE-FORMING PROTEIN	13.30- 13.40

4	Irina Shutova, The Institute of Bioorganic Chemistry NAS of Belarus, Minsk, Belarus PEPTIDES INHIBITOR OF THE BINDING OF ANTI-TPO AUTOANTIBODY: COMPUTER DESIGN, SYNTHESIS AND IN VITRO INVESTIGATIONS	13.40- 13.50
“Green Stay” Barbecue		14.00- 18.00
SESSION 7. ISTC		18.00 -19.30
Chairpersons: Tatyana Gremyakova, Nikolai Sokolov		
1	Nikolai Sokolov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia CONSTRUCTION OF ANTITUMOR STRAIN	18.00- 18.20
2	Nina Oparina Engelhardt Institute of Molecular Biology, RAS, Moscow, Russia UNSTABLE CHROMOSOMAL REGIONS IN THE HUMAN GENOME: COLON CANCER IMPLICATIONS	18.20- 18.35
3	Vladlen Skvortsov, Institute of Biomedical Chemistry, RAMS, Moscow, Russia THE MODEL OF TOXIC ACTION OF PROGESTERONE-LIKE CHEMICALS	18.35- 18. 55
4	Dmitry Druzhilovsky, Institute of Biomedical Chemistry, RAMS, Moscow, Russia COMPUTER-AIDED DISCOVERY OF NEW HIV-1 INHIBITORS	18.55-19.15
Gala Dinner		20.00 - 23.00

7 June 2008		
Breakfast		8.00 - 9.00
SESSION 8. SCI-MIX		9.00- 10.45
1	Samir Hanash, Fred Hutchinson Cancer Research Center, Seattle, USA NOVEL STRATEGIES TO INCREASE DEPTH OF ANALYSIS FOR BIOMARKER DISCOVERY USING PROTEOMICS	9.00- 9.30
2	Yuri Pankov, Institute of Experimental Endocrinology, Moscow, Russia DISTINCT PARTS OF POMC AMINO ACID SEQUENCE IN THE REGULATION OF FAT AND CARBOHYDRATE METABOLISM	9.40- 10.00
3	Irina Boksha, Mental Health Research Center RAMS, Moscow, Russia PROTEOMICS IN BIOLOGICAL PSYCHIATRY	10.00- 10.10
4	Olga Buneeva, Institute of Biomedical Chemistry, RAMS, Moscow, Russia PROTEOMIC IDENTIFICATION OF ISATIN BINDING PROTEINS	10.10- 10.20
CONCLUDING REMARKS, CLOSING CEREMONY		11.00- 12.30
Arrival to Moscow		13.00
Transportation to the Hotels and Airport		

SESSION I.

PROTEOMICS



WHAT CAN PROTEOMICS DELIVER TO MOLECULAR MEDICINE: EXAMPLE OF HEPATITIS C VIRUS AND ASSOCIATED LIVER DISEASES

Laura Beretta

Fred Hutchinson Cancer Research Center, Seattle, WA, USA

E-mail: lberetta@fhcrc.org

A trend of increasing rates of hepatocellular carcinoma has been reported worldwide, that is related to the high prevalence of hepatitis C virus (HCV) infection in the population. HCV often causes persistent infection in humans, a serious condition that is associated with chronic liver disease, cirrhosis and hepatocellular carcinoma, and represents a major issue of public health worldwide. For years, HCV research has been hampered by the lack of a robust cell culture system that recapitulates the complete viral life cycle. These limitations have been recently overcome by the use of the JFH genotype 2a strain of HCV, which permits propagation of infectious HCV particles in cell culture at high yields. We used this system to comprehensively characterize the protein changes associated with HCV infection and identify host proteins involved in HCV life cycle. We have also used a method to comprehensively and quantitatively profile the proteomes of the liver and plasma samples obtained from patients with chronic HCV infection and from mouse models of hepatocellular carcinoma. This method allowed for the identification of proteins specific to each disease stage and for the identification of protein abundance changes during progression from fibrosis to HCC, in both liver tissue and plasma. In conclusion, this method represents a promising approach for novel diagnostics and therapeutics discovery.

A COMPREHENSIVE STRATEGY FOR THE DISCOVERY AND VALIDATION OF HCC BIOMARKER

Keun Na, Eun-Young Lee, Hyoung-Joo Lee, An-Sung Jeong, Kwang-Youl Kim, Min-Seok Kwon, Hanna Lee¹, Sang Yun Cho, Hoguen Kim¹, and Young-Ki Paik
Yonsei Proteome Research Center, Department of Biochemistry and ¹Department of Pathology, Yonsei University College of Medicine, Yonsei University, Seoul, Republic of Korea
E-mail: paikyk@yonsei.ac.kr

N-linked glycosylation is an important target of clinical biomarker discovery, as evidenced by the fact that many biomarkers undergo this post-translational modification (i.e., alpha-fetoprotein, des-gamma-carboxyprothrombin, and glypican-3). To identify and characterize hepatocellular carcinoma (HCC)-specific N-linked glycoproteins, we used multi-lectin affinity chromatography to isolate intracellular glycoprotein fractions from five pairs of normal and HCC tissues. Two-dimensional differential gel electrophoresis, coupled with MALDI-TOF-MS analysis, revealed 54 differentially fluorescent protein spots, which included 12 unique single proteins with predicted glycosylated sites. One of these differentially expressed glycoproteins was YPRC-G1, a drug-metabolizing liver-specific enzyme. YPRC-G1 was remarkably down regulated in HCC tissues, a finding confirmed by western blot analysis and immunohistochemical staining of a tissue array containing non-tumor and cancerous tissue from 47 patients with stage I to IV HCC. We demonstrated, for the first time, that YPRC-G1 is expressed in human plasma, as determined by magnetic bead-based immunoprecipitation followed by nanoLC-MS/MS analysis with LTQ detection. Normalization of YPRC-G1 concentrations, as determined by this high-resolution proteomic approach, to whole protein levels, as determined by Western blot analysis, revealed that YPRC-G1 levels were 2.8-fold greater in plasma specimens from HCC patients (N=8) than in plasma from healthy volunteers (Student's *t*-test; $p < 0.01$). Thus, YPRC-G1 is a good candidate for further validation as HCC biomarker.

This study was supported by a grant from the Korea Health 21 R&D project, Ministry of Health and Welfare of Republic of Korea [A030003 to YKP].

PROTEOMIC MAPPING OF CYTOCHROMES P450 BY JOINING MASS-SPECTRAL INFORMATION FROM ADJACENT SLICES OF SDS-PAGE

Andrey V. Lisitsa, Natalia A. Petushkova, Irina I. Karuzina, Sergey A. Moshkovskii,
Alexander I. Archakov

V.N. Orekhovich Institute of Biomedical Chemistry, Russian Academy of Medical
Sciences, 119121, Pogodinskaya St., 10, Moscow, Russia

E-mail: andrey.lisitsa@ibmc.msk.ru

To discriminate between highly-homologous membrane proteins, such as cytochromes P450, a method of mass-spectral data processing was developed and implemented as web-software [1]. The method takes into account the mass-spectral information from adjacent slices of protein SDS-PAGE. SDS-PAGE gel was cut into thin (<200 μ m) slices and trypsin digest of each slice was processed in parallel by two mass spectrometry techniques, MALDI-TOF and LC-ESI-IonTrap MS/MS. Proteomic maps were constructed by assigning individual proteins to gel slices based on number of matching peptides in a corresponding MS-data. A total of 18 microsomal membrane proteins were mapped. These were 12 cytochrome P450 forms (CYPs 1B1, 4F2, 4A11, 1A2, 1A1, 3A43, 3A3/4/5, 2E1, 2A6/7/13, 2D6, 2C8, 2C9/10/19) and ATP synthase, UDP glycosyltransferase, flavin monooxygenase, carboxylesterase, epoxide hydrolase and actin. Pooling of mass spectrometric data, obtained from several adjacent gel slices, led to the 12 ± 5 % increase in protein sequence coverage. For example, joining of 4 slices increased sequence coverage of CYP2A protein up to 71%. This coverage sufficiently exceeded the 58 % level obtainable from the most informative single gel slice. It was also shown that in case of CYP3A family our approach enables to distinguish mass spectra associated with highly similar cytochromes P450 CYP3A3, 3A4 and 3A5.

[1]. <http://projects.ibmh.msk.su/oldzoomer/project/hlm2004/guest.pl>

INTERACTION OF OLIGOMERIC ENZYMES SUBUNITS IN SILICO AND IN VITRO

Alexis S. Ivanov, Andrey A. Molnar, Yury V. Mezentsev, Pavel V. Ershov,
Alexander I. Archakov

V.N. Orekhovich Institute of Biomedical Chemistry RAMS, Pogodinskaya str. 10, Moscow,
119121, Russia

E-mail: alexei.ivanov@ibmc.msk.ru

Currently protein-protein interactions (PPI) represent one of the key problem in proteomics. The contact areas in proteins complexes have unique structure and are very promising as targets for new generation of drugs [1]. The extremely attractive targets are the enzymes operating only in oligomeric complexes where the active sites are formed by residues from opposite subunits. The classic example of such enzyme is HIV protease (HIVp) which is active only in homodimeric form. We have used two oligomeric enzymes as test molecular objects that are the most convenient for PPI research - HIV-1 protease (homo-dimer) [2] and bacterial L-asparaginases (homo-tetramer) [3]. We have applied some computer methods (Amber 7 and Sybyl 6.9.1 software running on SGI Origin200 server), as well as direct PPI measurement by SPR-biosensor (Biacore-3000). Thermodynamics of subunits interaction in both enzymes, as well as interaction of HIVp dimerization inhibitors with HIVp monomers were also studied with optical biosensor.

The work was supported by Russian Foundation for Basic Research (grant 07-04-00575).

- [1] Veselovsky A.V. et al. (2002) Protein-protein interactions: mechanisms and modification by drugs. *J. Mol. Recognit.*, 15, 405-422.
- [2] Ivanov A.S., Gnedenko O.V., Molnar A.A., Mezentsev Y.V., Lisitsa A.V., Archakov A.I. Protein-protein interactions as new targets for drug design: virtual and experimental approaches. *J. Bioinform. Comput. Biol.* 2007, 5(2b), 579-592.
- [3] Mezentsev Yu.V., Molnar A.A., Gnedenko O.V., Krasotkina Yu.V., Sokolov N.N. and Ivanov A.S. Oligomerization of L-Asparaginase from *Erwinia carotovora*. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry.* 2007, 1(1), 58–67.

STEROID HORMONE ACTION ON THE PROTEOME STUDIED USING FISSION YEAST AS A MODEL

Susanne Bohmer*, Kyung Hoon Hwang*, Britta Wilzewski*, Christine Carapito**,
Emmanuelle Leize**, Alain Van Dorsselaer**, Rita Bernhardt*

*Universitt des Saarlandes, FR 8.3 Biochemie, P.O. Box 151150, D-66041 Saarbrucken,
Germany

**Laboratoire de Spectrometrie de Masse Bio-Organique, ECPM, 25, rue Becquerel, UMR
7509-CNRS/Universite Louis Pasteur, Strasbourg, France

E-mail: ritabern@mx.uni-saarland.de

The damaging effects of aldosterone on the human heart may partly be also mediated through non-genomic mechanisms. Our study concentrates on the mineralocorticoid receptor independent, non-genomic action of aldosterone and related hormones on protein level. We use the fission yeast *Schizosaccharomyces pombe* to study these effects. Fission yeast is unicellular and in many parameters closer to mammals than Baker's yeast and therefore represents a useful model organism. By using 2D-electrophoresis, we found 38 spots affected by aldosterone. These spots have been characterized using mass spectrometry techniques. Mass spectrometry analysis with MALDI-TOF MS and nanoLC-MS/MS approaches enabled the unambiguous identification of 11 proteins which were increased or decreased in their levels and which may represent new players and pathways of aldosterone-induced action. Two proteins with a connection to the osmotic regulation (NAD-dependent malic enzyme and glycerol-3-phosphate-dehydrogenase) as well as two proteins which are cohered to the overall organization of the cytoskeleton, vip1 and glyceraldehyd-3-phosphate-dehydrogenase, which was also found to be specifically affected by aldosterone in human HCT116 cells, are discussed. In addition to aldosterone, we also investigated the effect of its precursors, 11-deoxycorticosterone and corticosterone on the protein pattern to differentiate between specific effects of aldosterone on the protein pattern and more general effects of steroid hormones.

PLASMA PROTEOME PROFILING BY MALDI-TOF-MASS-SPECTROMETRY FOR CANCER DIAGNOSTICS

Sergei A. Moshkovskii, Maria A. Vlasova, Ilya Yu. Toropygin, Mikhail A. Pyatnitsky,
Alexander I. Archakov
Institute of Biomedical Chemistry, Moscow, Russia
E-mail: smosh@mail.ru

During recent seven years since this approach was suggested, the plasma proteome profiling by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass-spectrometry was used for discrimination between diseased and control biosamples. Originally implemented as a surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass-spectrometry the method used spectra as a blind barcode without knowledge about nature of discriminatory peaks. Consequently, many of such protein peaks were identified. In the present report, some aspects of discriminatory protein identity are discussed as well as a series of methods developed for such plasma proteome profiling. Using illustrative own data about identification of serum amyloid A as a discriminatory peak for human ovarian cancer, authors present their view on perspective of this approach in science and clinical practice.

PROTEOMICS REVEALS MOLECULAR TARGETS FOR ELIMINATION OF THERAPY-RESISTANT LEUKEMIA CELLS

Jan Zivny¹, Tereza Simonova¹, Ondrej Toman², Petr Halada³, Pavel Klener Jr.¹, and Jiri Petrak^{1,2}

¹ Department of Pathophysiology, 1st Faculty of Medicine, Charles University, Prague.

² Institute of Hematology and Blood Transfusion, Prague, Czech Republic

³ Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague

E-mail: jzivny@LF1.cuni.cz

Development of resistance to anti-cancer drugs is the major complication of chemotherapy. TRAIL (Tumor necrosis factor-related apoptosis-inducing ligand) is currently being tested as a new potential anti-cancer drug that induces apoptosis in many cancer cells including hematological malignancies. We used a proteomic approach to identify the “Achilles heel” of leukemia cells resistant to TRAIL that could serve as potential molecular targets for their selective elimination during a TRAIL therapy. By prolonged exposure to TRAIL (200 ng/mL) we derived two phenotypically distinct TRAIL-resistant leukemia cell subclones (P1, P2) from TRAIL sensitive HL-60 myeloid leukemia cells. We compared expression profiles of TRAIL-sensitive and TRAIL-resistant HL-60 cells using 2D-PAGE/MS analysis. In the two TRAIL-resistant HL60 leukemia subclones, we identified approximately 50 differentially expressed proteins compared to TRAIL-sensitive HL60 leukemia cell line. To identify potential therapeutic targets we focused our attention to non-structural proteins that showed decreased expression in TRAIL-resistant cells, namely two proteins essential for replication and DNA repair in P1 cells (MCM7 and RPA7) and adenosine deaminase in P2 cells. We hypothesized that decline of DNA repair/replication proteins, MCM7 and RPA7, can make the TRAIL-resistant P1 cells vulnerable to DNA damage and therefore sensitive to treatment with etoposide. Similarly, partial deficiency of adenosine deaminase can make the TRAIL-resistant P2 cells vulnerable to adenosine or vidarabine. We tested these hypotheses in series of *in vitro* experiments, using proliferation and apoptosis assay. The TRAIL-resistant and TRAIL sensitive cells were exposed to increasing doses of adenosine, vidarabine, and etoposide. Both hypotheses were confirmed, TRAIL-resistant P1 cells are highly vulnerable to etoposide, whereas TRAIL-resistant P2 cells are significantly more sensitive to adenosine or vidarabine, compared to TRAIL-sensitive cells and to the other TRAIL-resistant subclone. These findings are relevant for design of more effective strategies for leukemia therapy.

Support: MZCR/UHKT 023736, LC06044, MSM 0021621806, IGAMZ NR8317, NR8930.

PROTEIN FRACTIONATION AND RELATIVE QUANTITATION USING PF 2D AND ITRAQ FOR BIOMARKER QUEST

Suresh Jivan Gadher^{1,4}, Helena Skalnikova^{2,4} and Petr Halada^{3,4}

¹ Beckman Coulter International S.A., CH-1260 Nyon, Switzerland

² Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, v.v.i., 27721 Libečov, Czech Republic

³ Institute on Microbiology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

⁴ Joint Proteome Laboratory, Prague, Czech Republic
E-mail: sgadher@beckman.com

ProteomeLab™ PF 2D System – Protein Fractionation in 2 Dimension (Beckman Coulter, Fullerton, CA, USA) has been developed to fractionate complex protein mixtures by chromatofocusing in the first dimension followed by high-resolution non-porous silica reversed phase chromatography (RP LC) in the second dimension. Fractions of selected candidate proteins for biologically relevant qualitative and / or quantitative changes are then subjected to mass spectrometry to determine protein identity. Despite the high-resolution power of ProteomeLab™ PF 2D, UV-based quantitation could be compromised due to possible co-elution of several proteins into one fraction. Hence, we present an optimized protocol for application of isobaric tags for relative and absolute quantitation (iTRAQ) and MALDI-TOF/TOF mass spectrometry to obtain quantitative data from peptides derived by tryptic digestions of intact proteins fractionated by ProteomeLab™ PF 2D technique. To demonstrate the feasibility of such an approach, protein expression patterns obtained from the ProteomeLab™ PF 2D fractionation of human T-lymphoblastic leukemia CEM cell line were utilised. Untreated CEM cells (control) or CEM cells exposed to cyclin-dependent kinase inhibitor under experimental conditions were used as a source of protein samples. Differentially expressed protein fractions selected by UV quantitation in the ProteomeLab™ PF 2D system were further subjected to iTRAQ coupled to Mass Spectrometry. Based on the correlation between UV and iTRAQ quantitation, the potential biomarkers of anti-cancer activity of cyclin-dependent inhibition and development of chemoresistance were selected.

Complementary technologies may aid the quest for protein biomarkers. One such example is the processing of human follicular fluid (HFF) using ProteomeLab™ IgY partitioning system to reversibly capture abundant proteins from such human bio-fluids, yielding an enriched pool of low abundance proteins for further study. Decreased complexity of the starting material and access to masked proteins reflects a beneficial approach for complex starting materials. Such a complimentary approach was aimed at uncovering proteins typical for HFF and based on the criterion that many are found in significantly higher level in HFF compared to serum and /or plasma. Among them, clusterin, which is suggested to interact with components of the complement system and with inhibitory activity could be a potential candidate bio-marker. High expression of clusterin in HFF may be involved in protection of follicular environment from complement mediated damage during fertilization. Such potential candidate targets deserve further verification.

NEW APPROACHES FOR THE IDENTIFICATION OF PROTEINS INTERACTING WITH ENZYMES OF HEPATIC METHIONINE METABOLISM

Edel Reytor¹, Roberto Velasco^{1,2}, Rocío Vargas^{1,2}, Horacio Serrano⁴, Francisco Portillo¹, Dolores Pérez-Sala³, Jesús Vázquez⁴, Evgenij N. Nikolaev⁵, Igor A. Popov⁵, Aleksey S. Kononikhin⁵, María A. Pajares¹

¹Instituto de Investigaciones Biomédicas Alberto Sols (CSIC-UAM), Arturo Duperier 4, 28029 Madrid, Spain. ²FES Iztacala, Universidad Nacional Autónoma de México, México. ³Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040 Madrid, Spain. ⁴Centro de Biología Molecular Severo Ochoa (CSIC- UAM), 28049 Cantoblanco, Madrid, Spain. ⁵The Institute for Biochemical Physics Russian Academy of Sciences, Kosygina 4, 119334, Moscow, Russia.

E-mail: mapajares@iib.uam.es

Methionine adenosyltransferases (MAT) and betaine homocysteine methyltransferase (BHMT) are the enzymes of the mammalian methionine cycle in which our work has been focused. Several MAT isoenzymes are expressed in these organisms, MAT I/III in the adult normal liver, and MAT II in fetal liver and under pathological conditions. Approximately a 50% of the ingested methionine in humans is processed through this pathway that includes the only known reaction that generates S-adenosylmethionine (SAM), the main methyl donor in the cell. Under low levels of methionine the hepatocyte is able to resynthesize this amino acid using two enzymes, one of them, BHMT that allows recovery of one of the methylation equivalents used for choline synthesis. The last years have seen a number of reports that have shed light on the structure of these proteins, although important questions remain unanswered. Among them, the structure of MAT II hetero-oligomers and the contact surfaces between catalytic and regulatory subunit. Moreover, the abundance of MAT I/III and BHMT proteins in liver (~1% of the total protein) cannot be explained exclusively by their catalytic properties, and this fact together with their identification in unexpected locations, where no catalytic activity can be produced (eye lens lacks mitochondria, and hence betaine production) points to putative new roles for these proteins. Such roles may be exerted through protein-protein interactions for which scarce data are available, and hence our interest in finding putative candidates. For this purpose, several techniques have been used: yeast two hybrid, immunoprecipitation, affinity chromatography and two-dimensional gel electrophoresis. Putative targets have been identified through DNA and protein sequencing and the validity of these interactions is being confirmed *in vivo*. Comparison of the interaction patterns under control and pathological situations is also been carried out, and will assess potential novel roles for these enzymes. Possibilities of different mass spectrometry techniques, as well as ion fragmentation methods, were characterized as potential tools for revealing post-translational modifications in these enzymes.

APTAMER MULTIMERIC CONSTRUCTS – A NEW ROAD IN DEVELOPING SYNTHETIC AFFINITY REAGENTS FOR CHIP-BASED PROTEOMICS?

Sergey P. Radko, Nikolay V. Bodoev, Svetlana Yu. Rakhmetova, Oksana V. Gnedenko,
Alexis S. Ivanov, Alexander I. Archakov
Institute of Biomedical Chemistry, Russian Academy of Medical Sciences
E-mail: nikolai.bodoev@ibmc.msk.ru

A requisite condition for success in development of the biochip-based proteomics is an availability of highly affine and selective biorecognizing elements. Despite today's dominance of monoclonal antibodies (mAb) as capture reagents, there is an increasing interest to alternative reagents which could be more technologic, reproducible and cost-effective to substitute mAbs in a biochip development. RNA- and DNA-aptamers represent the most prominent alternative to mAbs with a great potential for protein detection in a biochip format. Being synthetically produced oligonucleotides, aptamers lend themselves to a molecular design. Aptamers recognizing distinct sites on a target protein can be combined into construct able to have simultaneous multiple interactions with the protein. Aptamers can easily be conjugated to each other either by merging aptamer motifs into a polynucleotide chain or by linking them with commercially available non-nucleotide spacers during chemical synthesis. Additionally to a "linear" arrangement, aptamers can be assembled into "branching" constructs via linkers containing sticky ends. Since the most affine binders are known to rely on multiple binding interactions, aptamer-based biorecognizing elements with enhanced affinity can be produced using linkers with a properly adjusted length and aptamers as building blocks. Different schemes to construct multimeric aptamer are considered. Design of aptameric constructs is illustrated with examples from the literature and own results on construction of anti-thrombin hetero- and homodimeric aptamers. Approaches to selection of aptamers recognizing different exosites on a target protein are discussed.

SESSION II.

GENOMICS



FUNCTIONAL GENOMIC STUDIES IN MICROBIAL CYTOCHROMES P450 (CYP)

Steve Kelly

Institute of Life Science and School of Medicine, Swansea University, Wales UK

E-mail: S.L.Kelly@swansea.ac.uk

Cytochrome P450 is not essential in bacteria but some have exhibited up to 1% of coding genes from this superfamily. In contrast eukaryotes normally have P450 that is essential for sterol biosynthesis plus a variety of other P450s that can constitute up to 1% of coding genes. Unravelling function is of academic and applied interest due to their functions in oxidative decoration of secondary metabolites and in detoxification of pollutants. The place of sterol biosynthetic P450 in the superfamily will be described together with discussion of point mutations in CYP51 in resistance mechanisms to the azole P450 inhibiting antifungal drugs. The multiplicity of actinomycete P450s in mycobacteria and streptomycetes will be described together with approaches to discovering function and new drug targets.

HOW SELENIUM HAS ALTERED OUR UNDERSTANDING OF THE GENETIC CODE

Xue-Ming Xu¹, Bradley A. Carlson¹, Vadim N. Gladyshev² and Dolph L. Hatfield¹

¹Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 USA,

²Dept of Biochemistry, University of Nebraska, Lincoln, NE 68588 USA

E-mail: hatfield@mail.nih.gov

Selenium is incorporated into protein as selenocysteine (Sec), the 21st amino acid in the genetic code, in response to the codon, UGA, which is normally used to signal the cessation of protein synthesis. Sec is a rare amino acid in protein and likely the only one that expanded the genetic code and has been preserved in all three domains of life. The biosynthesis of Sec occurs on its tRNA and its pathway of synthesis in eukaryotes was only recently determined through comparative genomics and experimental analyses in our laboratories. Sec tRNA is initially aminoacylated with serine by seryl-tRNA synthetase and the serine moiety is converted to phosphoseryl-tRNA by phosphoseryl-tRNA kinase. The phosphoseryl moiety is then hydrolyzed by Sec synthase (SecS) and the resulting product, while remaining attached to SecS, serves as the acceptor for the activated selenium donor, selenophosphate. Selenophosphate is synthesized by selenophosphate synthetase 2. Sec is then co-translationally inserted into protein in response to UGA with the help of a stem-loop structure in the 3'-untranslated region. The biosynthesis of Sec will be discussed along with the many health benefits of selenium. The benefits include roles in preventing cancer and heart disease, slowing the aging process and the onset of AIDS in HIV positive patients, inhibiting viral expression and roles in mammalian development, male reproduction and immune function. We devised several mouse models to elucidate the role of selenium, small molecular weight selenocompounds and selenoproteins in health and development. These models involve altering the expression of Sec tRNA either by developing mice carrying a standard or conditional knockout of the Sec tRNA gene, or wild type or mutant Sec tRNA transgenes. Generation of these mouse models and their usage in elucidating the role of selenium in health and development will be discussed.

HIGH-THROUGHPUT IDENTIFICATION OF CATALYTIC REDOX-ACTIVE CYSTEINE RESIDUES AND SELENOPROTEIN GENES

Vadim N. Gladyshev

Redox Biology Center and Department of Biochemistry, University of Nebraska, Lincoln, NE
68588 USA

E-mail: vgladyshev1@unl.edu

Cysteine (Cys) residues often play critical roles in proteins; however, identification of their specific functions has been limited to case-by-case experimental approaches. We describe a procedure for large-scale detection of catalytic redox-active Cys through homology to sporadic selenocysteine (Sec)-containing proteins. This method is not dependent on sequence motifs, structure and origin of the sequences and first identifies unique Cys/Sec pairs flanked by homologous sequences within the universe of translated nucleotide sequences; these pairs then serve as seeds for sequence analysis at the level of protein families and subfamilies. A variation of this method allows identification of selenoprotein genes in sequence databases. Application of this method identified majority of known proteins containing catalytic redox-active Cys, while filtering out proteins in which conserved Cys are involved in other functions, such as non-redox catalysis, structural disulfides, posttranslational modifications and binding of certain metals. Moreover, for oxidoreductases containing multiple conserved Cys, the identity of the attacking catalytic Cys could be identified. We predicted redox-active Cys in several proteins, and directly verified the prediction in an S-adenosyl methionine-dependent methyltransferases family. Also presented will be characterization of selenoproteomes in a variety of organisms and evolution of selenoproteomes and thiol oxidoreductases. Rapid accumulation of sequence information from genomic and metagenomic projects should allow detection of many additional oxidoreductase and selenoproteins families as well as identification of redox-active Cys and Sec in these proteins.

- [1] Fomenko, D. E., Xing, W., Adair, B. M., Thomas, D. J., and Gladyshev, V. N. (2007) High-throughput identification of catalytic redox-active cysteine residues. *Science* **135**, 387-389.
- [2] Kryukov, G. V., Castellano, S., Novoselov, S. V., Lobanov, A. V., Zehtab, O., Guigo, R., and Gladyshev, V. N. (2003) Characterization of mammalian selenoproteomes. *Science* **300**, 1439-4313.

DIAGNOSTIC PYRAMID FOR BACTERIAL IDENTIFICATION AND TYPING

Elena N. Ilina

Research Institute of Physico-Chemical Medicine,
Malaya Pirogovskaya, 1a
119992, Moscow, Russia
E-mail: ilinaen@gmail.com

Development and introduction of new adequate systems for microbial identification and typing remain the actual problem of medical associated investigations. Nowadays there are three hotspots in the field of clinically relevant microorganism studies – creation of molecular techniques for microbial species identification, for genetically determined drug-resistance discovery and for molecular epidemiology monitoring. All of them concerned the problems of drug-resistance monitoring and prevention of pathogenic microbial strains spreading. During the last years such kind systems based on MALDI-ToF mass spectrometry for molecular monitoring of *M. tuberculosis*, *S. pneumoniae* and *N. gonorrhoeae* clinical strains have been developed. High genetic and proteomic heterogeneity for certain microorganism large group collected from different part of Russia were revealed. Followed comparative analysis obtained data led to several practically and scientifically important findings. Thus the possible of streptococci mitis group discrimination by MALDI-ToF mass spectrometry based direct bacterial profiling was shown. Comparison of genetic markers distribution in particular susceptibility groups allowed to select the nucleotide alterations or their combinations significantly associated with drug resistance of *N. gonorrhoeae* and *S. pneumoniae*. Moreover a new possible way of drug resistance development was suggested by further proteomic investigation of a few *N. gonorrhoeae* strains revealed discordance between genetic analysis and susceptibility testing. So the diagnostic pyramid being created for bacterial identification and typing appears a powerful tool for scientific research of microbial cells organization and functioning.

MALDI-TOF MASS-SPECTROMETRY AS A TOOL FOR ANALYSIS OF GENOMIC SEQUENCE POLYMORPHISMS. HIGH-THROUGHPUT SCREENING OF GENETIC ALTERATIONS IN RUSSIAN COLORECTAL CANCER PATIENTS

Edward V. Generozov, Petr A. Kostin, Tatyana V. Pogoda, Vadim M. Govorun
Research Institute of Physico-chemical medicine,
Malaya Pirogovskaya, 1a
119992, Moscow, Russia
E-mail: generozov@gmail.com

DNA variation analysis enters a new stage of research. Many projects in current human genetics aim to dissect complex traits by making use of DNA markers, mainly by single nucleotide polymorphisms (SNPs) or another DNA variations. From the point of view of applied medicine, an actual problem is large-scale population screening to estimate the allele frequencies of medical-significant genetic alterations associated with the risk of development multifactorial diseases. One of the modern schemes for SNP determination is DNA mini-sequencing method followed by the detection of the reaction products by MALDI-TOF mass-spectrometry. Obvious advantages of such combination include accuracy of the analysis, high efficiency and absence of isotopic or fluorescent markers. These and other factors determine the low prime cost per reaction. Colorectal cancer (CRC) is one of the most increasing cancers in Russia. In a pilot study we employed a MALDI-TOF mass-spectrometry approach to genotyping genomic and somatic mutations in clinical samples obtained from Russian CRC patients, colon adenoma (CA) patients and inflammatory bowel disease (IBD) patients as well as in patients of control group. Results of genomic scanning reveal a significant difference in distribution of IL10 gene promoter region SNPs in the groups under study ($\chi^2=6.265$, $P=0.0025$). The somatic mutation in TP53, APC, k-RAS and BRAF genes was discovered by MALDI-TOF minisequencing of DNA isolated from colon tissues. Correlations between genetic markers and clinical factors were analyzed after reviewing medical records. The positive rates for alterations of APC, K-ras and TP53 in cancer tissue samples were 44%, 24%, and 17%, respectively. APC mutations were frequently noted in early-stage cancer, whereas TP53 and k-RAS was observed mainly in developed cancers. Obtained results demonstrated the MALDI-TOF genotyping as appropriate and reliable approach for analysis of germline as well as somatic DNA variations.

FULL GENOME TRANSCRIPTOM ANALYSIS OF T-REGULATORY CELLS ISOLATED FROM CANCER PATIENTS

Alexei Yu. Lupatov^{1,2}, Leonid K. Kurbatov¹, Pavel A. Karalkin^{1,2}, Ivan B Cheglakov¹,
Konstantin N Yarygin^{1,2}

¹Institute of Biomedical Chemistry of the Russian Academy of Medical Science, 10 Pogodinskaya Str., 119121 Moscow, Russia;

²Russian State Medical University, 1 Ostrovityanova Str., 117997 Moscow, Russia.

E-mail: lupatov@ibmc.msk.ru

Activation and expansion of the CD4⁺CD25⁺ T-regulatory cells is presently regarded as a key mechanism of tumor escape from immunological control. Unfortunately, no reliable protein markers of those cells have been identified yet. The goal of this work was to carry out the gene expression analysis of CD4⁺CD25⁺ T-regulatory cells and CD4⁺CD25⁻ T-helper cells in an attempt to find differentially expressed genes. We found a substantial (2-5 folds) increase of the CD4⁺CD25⁺ T-regulatory cell numbers in the peripheral blood of renal cell carcinoma and colon cancer patients. CD4⁺CD25⁺ cells and CD4⁺CD25⁻ cells were isolated from the peripheral blood mononuclear cell fraction of patients with renal cell carcinoma or colon cancer using combined positive and negative selection with immunomagnetic beads labeled with specific antibodies. Activated CD25⁺ effector T lymphocytes were excluded by the negative selection with beads conjugated to the anti-CD45RA antibodies. Positively selected CD4⁺CD25⁺ cells were sorted using the BD FAXAria cell sorter in order to isolate the CD127⁻ subpopulation exhibiting the highest regulatory activity in vitro. RNA isolated from the resulting CD4⁺CD25⁺ and CD4⁺CD25⁻ cells was analyzed using the Agilent Whole Human Genome microarray. The expression levels of 37 genes were 2 times or more higher in the former compared to cells with the CD4⁺CD25⁻ T-helper phenotype. The list contained several known human regulatory cell markers including *CD25*, *CTLA4*, *FOXP3*, *CCR7*, and *TNFRSF1B* while some up-regulated genes have never been previously associated with the T-regulatory cell phenotype. One of them (*A_24_P938583*) has high similarity with *TDP43* regulatory gene that exhibits DNA binding capacity.

This work partially supported by grant #02.512.11.2063 from the Russian Federal Agency of Science and Innovations.

ANALYSIS OF GENETIC SUSCEPTIBILITY FOR BRONCHIAL ASTHMA IN RUSSIANS

Olga E. Voronko, Elena V. Dmitrieva-Zdorova, Nikolai V. Bodoev, Alexander I. Archakov
Research Institute of Biomedical Chemistry RAMS, 119121 Pogodinskaya str., 10 Moscow,
Russian Federation
E-mail: vr_olga@yahoo.com

Asthma is complex genetic disease which pathogenesis is a mix of environmental and genetic factors lead to chronic airway inflammation and subsequent manifestation of clinical disease. The majority of genetic studies in the field have concentrated on identification of genes increasing susceptibility to the disease initiation and progression. We have used 19 polymorphic markers of 13 candidate genes (*IL4RA*, *IL5*, *IL13*, *IL18RA*, *FCER1B*, *CTLA4*, *TLR4*, *CARD15*, *CCL26*, *GPR4*, *CC16*, *CCL5*, *ADAM33*) to study association of these genes with atopic bronchial asthma in Russian patients. 283 patients with atopic bronchial asthma and 180 healthy individuals were examined. The genotyping was performed by MALDI-TOF mass spectrometry. For 11 markers we have found statistically reliable association with asthma and different aspects of asthma development. Our results could be helpful for both better understanding of the pathogenesis of asthma and identification of predictors of disease progression.

SESSION III.

BIOINFORMATICS



BIOINFORMATICS STRATEGIES FOR DIAGNOSIS AND BIOMARKER DISCOVERY IN MALDI-TOF MS PROFILES

Herbert Thiele

Bruker Daltonik GmbH

Fahrenheitstrasse 4, D 28359 Bremen

E-mail: ht@bdal.de

With new applications of mass spectrometry in the field of clinical proteomics pattern recognition and classification techniques gain increasing importance. This is especially true for the analysis of MALDI MS spectra of body fluids. Automatic classification of high-resolution mass spectrometry proteomic data has increasing potential in the early diagnosis of cancer and is essential for a successful treatment. A new data processing pipeline for biomarker discovery in serum protein profiles is demonstrated.

The analysis of such high dimensional data is complex. Hence, processing of the spectra and algorithms for the supervised and unsupervised analysis of the extracted features has to be reconsidered. Thereby, especially the usage of wavelet coefficients to improve the preprocessing and the extracted features is promising. First an initial preprocessing of the whole set of spectra is performed. In particular the baseline by means of the discrete bi-orthogonal wavelet transform is removed. This step is followed by the discrete wavelet transformation (DWT) of the spectra for feature extraction. DWT is a major tool in signal processing, in particular for its superior properties of denoising and compression. The central idea of DWT is to find a lossless multi-scale representation of data by means of wavelet coefficients. The obtained approximation coefficients exhibit a high peak pattern matching property and feature a denoising of the spectrum [1]. In order to find those wavelet coefficients which express differences between cancer and control spectra, a nonparametric statistical testing of the null hypothesis H_0 of no distinction between cancer and control groups expressed by the i 'th coefficient is used (Kolmogorov-Smirnov test). The selected discriminative DWT coefficients are used as input data for classification by Support Vector Machines (SVM) of type C-SVM with the gaussian kernel. SVM is a powerful and popular machine learning technique, widely used for classification and extensively applied in biology [2]. The main idea of SVM is establishing a maximal margin classifier. Linear in its trivial formulation, SVM classifier becomes non-linear by exploiting a kernel function or a kernel. For the choice of SVM hyperparameters the double cross-validation (CV) paradigm is used [3]. Double CV is a bias-reducing scheme of simultaneous parameters estimation and assessment of the classifier with these parameters.

The proposed processing pipeline is assessed using MALDI-TOF serum protein profiles of 64 colorectal cancer patients and 48 controls. The procedure provided almost perfect total recognition rate (97.3%), sensitivity (98.4%), and specificity (95.8%). In the main, the extracted biomarkers reproduce the distinguishing peaks but the procedure allows to extract information that can not be easily derived by simple comparison of the spectra. The obtained classifiers have good generalization properties which prevents overfitting to the given data and leads to reproducibility of results.

- [1] Schleif, F.M. et.al.; Comput. Visual Science; Springer Verlag 2008.
- [2] De Noo, M.E. et.al.; Onkologie 2006;29;501-506.
- [3] Mertens, B.J.A. et.al.: J. Computational Biology; 13 (9), 1591-1605, 2006.

CLASSIFICATION OF TRIPLET PERIODICITY IN DNA SEQUENCES OF KNOWN GENES TAKEN FROM KEGG DATABANK

Felix F. Frenkel, Eugene V.Korotkov

Bioengineering Centre of RAS, 60-letiya Oktyabrya prosp., 7/1, Moscow 117312, Russia

E-mail: genekorotkov@gmail.com

We introduce a new concept of triplet periodicity class and a measure of similarity between such classes. We performed classification of 472288 triplet periodicity regions found in 578868 genes from 29th release of KEGG databank. Totally 2520 classes were obtained. They contain 94% of 472288 found cases of triplet periodicity. For 92% of triplet periodicity regions contained in classes the same linkage of triplet periodicity to reading frame is observed. For 8% of triplet periodicity cases we revealed a shift between reading frame of a gene and reading frame common for majority of genes contained in a class of triplet periodicity. For these 8% of periodic regions the hypothetical amino acid sequences corresponding to reading frame built by triplet periodicity class were made. BLAST program has shown that 2679 hypothetical amino acid sequences have statistically significant similarity with proteins from UniProt databank. We suppose that 8% of triplet periodicity regions contained in classes possess a mutation originating from reading frame shift. Obtained classes of triplet periodicity can be used for identification of genes' coding regions as well as for searching for mutations arisen from reading frame shift.

1. Korotkov, E.V., Korotkova, M.A., Kudryashov, N.A. 2003b. Information decomposition method for analysis of symbolical sequences. *Physics Letters A* 312(3-4): 198-310.
2. Korotkov, E.V., Korotkova, M.A., Frenkel, F.E., Kudryashov, N.A. 2003a. The informational concept of searching for periodicity in symbol sequences. *Molekuliarnaia biologiya* 37(3): 436-451.

VIRTUAL SCREENING OF INHIBITORS OF HIV PROTEASE DIMERIZATION USING MM-PBSA METHOD

Alexander V. Veselovsky, Vladlen S. Skvortsov
V.N.Orekhovich Institute of Biomedical Chemistry RAMS, Moscow
10, Pogodinskaya str., 119121, Moscow, Russia
E-mail: veselov@ibmh.msk.su

Human immunodeficiency virus type-1 protease (HIV-1-PR) is an essential enzyme in the viral life cycle, and a major target for therapeutic intervention. The HIV-1 protease is composed of 99 amino acids and is a member of the family of aspartic acid proteases. Unlike the cellular aspartic proteases that are active as monomers, catalytic activity of retroviral proteases including HIV-1 protease requires dimer formation. At present about 10 inhibitors of HIV-1-PR are in clinic practice. But drug resistance has severely limited the effectiveness of HIV-1-PR inhibitors in AIDS therapy. One way for overcome the drug resistance is the design of inhibitors of protein-protein interaction (PPI). PPI is more “resistance” for spontaneous mutations at their binding site since high conservatism of amino acid residues of protein-protein interfaces. The aim of our investigation was found compounds that used or in clinic trial that can interact with HIV-1-PR dimer interfaces. For this purpose molecular database CMC (MDL) was used. The target for virtual screening was monomer of HIV-1-PR. Ten snapshots of this monomer from molecular dynamics were selected. Molecular docking of compounds from database was done in each snapshot of HIV-1-PR monomer. Primary selection of complexes was done by Dock 6.0 scoring function. The free energy of protein-ligand complexes of top compounds was predicted using Poisson-Boltzmann/surface area (MM-PBSA) methods. It allows to take into account steric, electrostatic forces and solvation free energy. Several compounds were selected for experimental testing for ability to interact with HIV-1-PR dimer interfaces.

ENOLASE AND HEAT SHOCK PROTEINS AGAIN? THE FIRST PROTEOMIC HIT PARADE OF REPEATEDLY IDENTIFIED DIFFERENTIALLY EXPRESSED PROTEINS

Jiri Petrak^{1,2}; Robert Ivanek³; Ondrej Toman¹; Daniel Vyoral^{1,2}; Jan Zivny²

¹Institute of Hematology and Blood Transfusion, Prague, Czech Republic

²Department of Pathophysiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

³ Institute of Molecular Genetics, Academy of Sciences, Prague, Czech Republic

E-mail: petrak@uhkt.cz

Conventional two-dimensional electrophoresis (2-DE) remains a fundamental tool in expression proteomics. However, after reading several 2-DE-based articles featuring lists of identified differentially expressed proteins, one starts experiencing a disturbing sense of *děj* vu. Heat-shock proteins again? Elongation factors, proteasome subunits or peroxiredoxins once more? The same proteins seem to predominate regardless of the experiment or tissue. To explore this phenomenon and to quantify the occurrence of individual differentially expressed proteins in 2-DE studies, we compiled identities of proteins from 2-DE experiments published in 3 recent volumes of PROTEOMICS. We then calculated the appearance of the most predominant proteins in the dataset and assembled the „TOP 15“ charts for the individual proteins and protein families. The “TOP 15” charts for individual proteins demonstrate that the most frequently identified differentially expressed protein in rodents is enolase 1. In humans, HSP27 is the most notoriously identified protein, closely followed by enolase 1. Enolase 1 was identified as differentially expressed in about 30 percent of all published experiments in both human and rodent tissues. The “TOP 15” charts for protein families indicate that keratins and peroxiredoxins are the most often differentially expressed protein families in human and rodent samples respectively. Results of our meta-analysis confirm that some proteins appear among the identified differentially expressed proteins very often, regardless of experiment or tissue. Are we observing universal cellular sensors or rather technical artifacts and limitations of the 2-DE technology? Why should a conserved glycolytic enzyme such as enolase1 change its expression in response to so many different stimuli? Why are differentially expressed keratins identified more often in human samples than in rodent tissues? Our study demonstrates that meta-studies of published proteomic data could provide invaluable information pertinent to the biological processes or the methods involved. We believe that our “TOP15” charts could help prevent over-interpretation of 2-DE-based results and focus our attention to more specific and more relevant proteins.

Supported by MSMT CR - LC06044, 0021621806 , MZCR/UHKT 023736 and by IGA MZ NR8317.

PDB LIGANDS, SITES AND MOTIFS DATABASE WITH THE SEARCH ENGINE

Adel Golovin and Kim Henrick

EMBL Outstation, The European Bioinformatics Institute, Wellcome Trust Genome Campus,
Hinxton, Cambridge, United Kingdom

E-mail: golovin@ebi.ac.uk

We describe a comprehensive, freely available on the web PDB search tool for biomolecule crystallographers, biochemistry students, scientists and researchers. The search database is an integrated resource, which provides information about ligands, protein sequence and structure motifs, their relative position and the neighbour environment. The details are derived from the PDB together with a mapping to other motif and active-site sources. All data are stored in a relational database, accessible through the interactive service for fast search and visualization capabilities. Search criteria can combine ligands, sequence motifs, structure motifs, protein sequences, 3D properties (like ϕ/ψ and Ω angles, CA and side-chain positions), sequences secondary structure elements, 3D associations of small motifs, protein side-chain and main-chain bonds and protein-ligand interactions. We also offer different views on the data and provide multiple sequence and multiple structure alignment tools. Binding statistics over PDB archive with Relative Risk (RR) distribution gives an insight into protein features. Protein sequence and structure motifs have an application in drug design where motifs map to active-sites and ligand binding sites. Each process in a computer aided drug design is iterative and can be represented as a loop of search, alignment, modelling, prediction, analysis where the search helps to find a good prototype for further modelling. It provides data and hints for multiple alignment. Recent research results have shown that the multiple sequence and 3D structure alignment is essential for modelling where it substantially improves the quality and accuracy.

TEXTOMICS TOOLS FOR AUTOMATICALLY UPDATE THE HIT-PARADE OF REPEATEDLY IDENTIFIED PROTEINS

Elena A. Ponomarenko¹, Andrey V. Lisitsa¹, Jiri Petrak^{2,3}, Yuliana V. Miroshnichenko¹,
Alexander I. Archakov¹

¹ Institute of Biomedical Chemistry of RAMS. Pogodinskaya 10, 119121 Moscow, Russia

² Institute of Hematology and Blood Transfusion, U Nemocnice 1, Prague 2, Czech Republic

³ Department of Pathophysiology, First Faculty of Medicine, Charles University, U
Nemocnice 5, Prague 2, Czech Republic

E-mail: pon@ibmh.msk.su

It was recently shown that meta-analysis of published proteomic articles reveals a set of 15 most predominant proteins (TOP 15), which are identified as differentially expressed regardless of experiment, tissue of species [1]. Here we demonstrate that repeatedly identified proteins are also the most frequently mentioned protein names in the texts of proteomics articles. Using such feature we can automatically update the "Hit parade of repeatedly identified differentially expressed proteins". We retrieved about 30 thousands names including synonyms of human proteins from UniProt database (www.beta.uniprot.org). Retrieved items were searched in full-text articles: we used the same articles, that have been previously used to compile TOP15 (2-DE based articles published in volumes No. 4-6 of Proteomics). Among most frequently occurring protein names (occurring more than 10% articles) identified by our automatic text analysis we found 13 matches with the original TOP15 chart (that was compiled manually from result tables in published articles). The match rate becomes worse (9 out of TOP15) if abstracts are used instead of full texts. Also, if we diminished number of alternative protein names (e.g., if we use only recommended protein name), we rarely retrieved more than 4 proteins matching the TOP15. We conclude, that in presently UniProt includes protein names which are suitable for meta-analysis of proteomic data. Therefore collections of disease-related proteomic articles can be used for first step in automatic selection of potential biomarkers.

[1]. Petrak J, Ivanek R, Toman O, Cmejla R, Cmejlova J, Vyoral D, Zivny J, Vulpe CD. Déjà vu in Proteomics. A hit parade of repeatedly identified differentially expressed proteins. *Proteomics*. 2008 May;8(9):1744-9.

METHOD OF PERSPECTIVE TARGET ANALYSIS FOR BREAST CANCER THERAPY

Olga N. Koborova, Dmitry A. Filimonov, Alexey V. Zakharov, Alexey A. Lagunin and Vladimir V. Poroikov

Institute of Biomedical Chemistry of Russian Academy of Medical Sciences, Pogodinskaya 10, Moscow, 119121, Russia;
E-mail:okoborova@gmail.com

One of the most important problems in search for new anticancer therapies is identification of proteins that are involved in emergence and progression of malignant diseases, and to find the most prospective targets among them.

We propose an algorithm of anticancer drug target identification. The algorithm models networks of cell cycle regulation as logic networks. Input data is the beginning states of nodes (proteins and/or genes) in a primary moment of time. Output data is a number of node states in different time moments – trajectories. For analysis of protein influence that can change trajectories in a desired way (for example, apoptosis stimulation of cancer cells without influence on normal cells or stop of cell cycle progression), the node states can be fixed according to the type of proposed effect, for example, inhibition of the respective protein(s).

The method was applied to the case of breast cancer using molecular network, which includes such pathways and their interconnections as hedgehog, Wnt/ β -catenin, VEGF, IL-6, TNF α and some other pathways which are known as important pathways in breast cancer progression. The most of pathways were taken from TRANSPATH database (<http://www.biobase.de>) and partly from recent publications, and expression data, consisting of up and down regulated genes list, were taken from Cyclonet database (<http://cyclonet.biouml.org>).

Two groups of promising targets were identified: first - a set of targets, which inhibition changes trajectory into interruption of cancer cells division and second - a number of targets, which inhibition changes trajectory into apoptosis of breast cancer cells.

The work was supported by European Commission project No. 037590 (FP6-2005-LIFESCIHEALTH-7).

PREDICTION OF FUNCTIONALLY RELATED PROTEINS: PHYLOGENETIC PROFILES AND CLUSTER ANALYSIS

Mikhail A. Pyatnitskiy, Andrey V. Lisitsa, Alexander I. Archakov
Institute of Biomedical Chemistry, Russian Academy of Medical Sciences,
Pogodinskaya Str., 10, Moscow, 119121, Russia
E-mail: mpyat@ibmh.msk.su

The advent of whole-genome sequencing has led to computational methods that infer protein function and linkages. One of the most promising approaches for prediction of protein-protein structural and functional interactions is studying of phylogenetic profiles. A phylogenetic profile of a protein is binary vector, representing the presence or absence of homologs to that protein across a set of organisms. It was shown that proteins with similar patterns of co-occurrence across many organisms tend to participate in the same protein complex, biochemical pathway or have similar sub-cellular location. In the present work we explored the application of cluster analysis to phylogenetic profiling in order to improve performance of the method. We applied several standard techniques of cluster analysis including different versions of hierarchical clustering, k-means, PAM. We also used probability for phylogenetic profiles to coincide by chance as a measure of distance. We chose *E.coli K12* as a target organism; different sets of reference genomes were tried. We proposed use of distance between different clusterings as a measure of method performance. One clustering is golden truth (reference database like KEGG or GO) and the other is result of clustering of phylogenetic profiles. This approach allowed us accurately compare different aspects of an algorithm. By comparing resulting predictions to expert annotations in KEGG database we obtained that the most accurate methods were complete hierarchical clustering and Ward's method. This results lead to improved version of phylogenetic profiling method and hence, more precise prediction of functionally related proteins.

LARGE DATABASE RESOURCES AND WEB TOOLS FOR DRUG DEVELOPMENT

Igor V. Filippov, Markus Sitzmann and Marc C. Nicklaus
Computer-Aided Drug Design (CADD) Group, Lab. of Medicinal Chemistry, Center for
Cancer Research, National Cancer Institutes, National Institutes of Health, DHHS. NCI-
Frederick, Bldg. 376, 376 Boyles St., Frederick, MD 21702, USA.
E-mail: mn1@helix.nih.gov

We present new tools and services developed by the CADD Group, NCI, NIH, in the context of our chemoinformatics and drug development work, made available on the CADD Group's web site <http://cactus.nci.nih.gov>. These tools are designed for looking up structures in very large databases of small molecules, for creating and recognizing chemical structure drawings, and for generation and interpretation of chemical identifiers. One of our services is a web site for very rapid structure lookup in an aggregated collection of currently more than 52 million entries from 80 databases, comprising more than 36 million unique structures. This Chemical Structure Lookup Service (CSLS) contains toxicology-related databases, catalogs of commercially available samples, drugs, assay results data sets, and databases in several other categories. CSLS allows the user to find out very rapidly in which one(s) of all these databases a given structure occurs independent of the representation of the input structure, by making use of InChIs and InChIkeys as well as of the CACTVS hashcode-based identifiers developed in our group. These new, calculable, identifiers are designed to take into account tautomerism, different resonance structures drawn for charged species, and presence of additional fragments. They make possible fine-tunable yet rapid compound identification and database overlap analyses. We also present an open-source Optical Structure Recognition Application (OSRA), which can be used as a downloadable software tool, or via implementation as a web service on our server.

BIO- AND CHEMINFORMATICS APPROACHES TO DISCOVERY OF NEW ANTINEOPLASTIC TARGETS AND LIGANDS

Vladimir Poroikov, Dmitry Filimonov, Alexey Lagunin, Tatyana Glorizova

Institute of Biomedical Chemistry of Rus. Acad. Med. Sci.; 10, Pogodinskaya Street,
Moscow, 119121, Russia;
E-mail: vladimir.poroikov@ibmc.msk.ru

Due to the progress in postgenomic studies it became obvious that many diseases have a complex etiology. The multitargeted drug concept appears, according which such remedies might have some advantages comparing to the monotargeted medicines.

Discovery of new multitargeted drugs can be made by prediction of biological activity spectra with computer system PASS (<http://www.ibmc.msk.ru/PASS>), which predicts about 3,300 pharmacotherapeutic effects, biochemical mechanisms of action, specific toxicities and metabolic terms with average accuracy about 94% (leave-one-out cross-validation). About 9000 compounds from the total PASS training set exhibit antineoplastic activity caused by more than 200 molecular mechanisms of action.

Potential of bioinformatics and computer-aided drug discovery methods in identification of prospective sets of particular molecular targets and finding of lead substances for future antineoplastic multitargeted drugs in the databases of available chemical compound samples will be discussed.

This work was supported by FP6 (LSHB-CT-2007-037590 - Net2Drug, RFBR (05-07-90123, 06-03-08077, 06-03-39015).

SHAPE, FLEXIBILITY, REACTIVITY: A MOLECULAR MODEL OF REGIOSELECTIVITY OF CYTOCHROME P450 MONOOXYGENASES

Alexander Seifert, Ricardo Branco, Demet Sirim, Stephan Tatzel, Jurgen Pleiss

Institute of Technical Biochemistry, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany;

E-mail: Juergen.Pleiss@itb.uni-stuttgart.de

Cytochrome P450 monooxygenases (CYPs) are ubiquitous heme-containing enzymes which selectively catalyze a wide variety of oxidative reactions with a broad substrate spectrum. CYPs consist of conserved regions that are essential for structure and function, and of variable regions that mediate the individual biochemical properties. To investigate the structural basis of specificity and regioselectivity, two approaches were combined: a systematic comparison of sequences and structures to derive simple rules for predicting the regioselectivity of CYPs, and molecular modelling of substrate binding to investigate the effect of shape and flexibility.

The Cytochrome P450 Engineering Database was established to serve as a tool for a comprehensive and systematic comparison of CYPs.¹ It currently integrates sequence and structure data of 3911 and 25 proteins, respectively. Proteins are grouped into homologous families and superfamilies according to Nelson's classification. Functionally relevant residues are annotated. The web accessible version contains multisequence alignments, phylogenetic trees, and HMM profiles.

To further investigate the molecular basis of regioselectivity, multiple molecular dynamics simulations of enzyme-substrate complexes were performed. High mobility of the substrate binding pocket allows the enzyme to adapt for substrates of different size and shape, which is consistent with the broad substrate profile observed for CYPs. On the other hand, amino acids in the rigid core of the enzyme surrounding the active site are forming a narrow funnel that allows the substrate to reach the active heme oxygen in selected orientations only, resulting in a well-defined regioselectivity.² For the human CYP2C9 and the bacterial CYP102A1, selectivity-determining sites in the binding sites were identified. For CYP102A1, mutants with changed regioselectivity towards fatty acids and monoterpenes were predicted.³ A comparison of MD simulations on different mutant-substrate complexes provides a detailed picture of the effect of shape and side chain flexibility to the orientation of a substrate, and allows the prediction of regioselectivity from first principles.

1. Fischer M, Knoll M, Sirim D, Wagner F, Funke S, Pleiss J. The Cytochrome P450 Engineering Database: a navigation and prediction tool for the cytochrome P450 protein family. *Bioinformatics* 2007;23:2015-2017.
2. Seifert A, Tatzel S, Schmid RD, Pleiss J. Multiple molecular dynamics simulations of human p450 monooxygenase CYP2C9: the molecular basis of substrate binding and regioselectivity toward warfarin. *Proteins* 2006;64:147-155.
3. Branco RJF, Seifert A, Budde M, Urlacher VB, Ramos MJ, Pleiss J. Anchoring effects in a wide binding pocket: the molecular basis of regioselectivity in engineered cytochrome P450 monooxygenase from *B. megaterium*. *Proteins: Structure, Function, and Bioinformatics* (in press)

RECOGNITION OF PROTEIN FUNCTION USING THE LOCAL SIMILARITY

Kirill E. Alexandrov, Dmitry A. Filimonov, Boris N. Sobolev, Vladimir V. Poroikov
Institute of Biomedical Chemistry, Russian Academy of Medical Sciences,
Pogodinskaya Str., 10, Moscow, 119121, Russia
E-Mail: dmitry.filimonov@ibmc.msk.ru

Functional annotation of amino acid sequences is one of the most important problems of bioinformatics. Different approaches were applied for recognition of some functional classes; nevertheless many functional groups still cannot be predicted with the required accuracy. We developed a new method of the protein functions' recognition based on the original concept of local similarity. The query ("new" sequence) is compared with each sequence from the set of sequences with known functions; the local similarity scores are calculated for the query sequence positions and used as input data for classifier. The method was tested on different sequences' sets. About 100% recognition accuracy was obtained at various levels of classification hierarchy for two sets of proteins represented non-intersected functional classes. Prediction accuracy was lower (but still reasonable) for recognition of ligand specificity in cytochromes P450. The performance of the proposed method is rather high, and it can be successfully applied for both prediction of protein functional classes and selection of functionally significant sites in amino acid sequences.

LABORATORY INFORMATION MANAGEMENT SYSTEM “PROTEY”

Yuriy O. Koptsov, Irina I. Karuzina, Natalya A. Petushkova, Andrey V. Lisitsa,
Vlad Rudenko

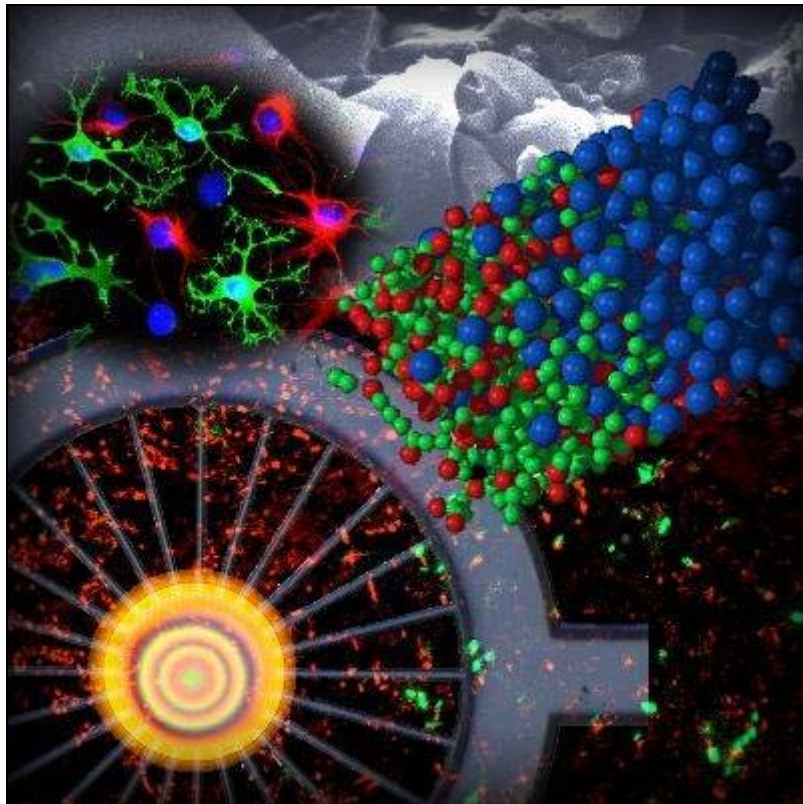
Institute of Biomedical Chemistry, Russian Academy of Medical Sciences,
Pogodinskaya Str., 10, Moscow, 119121, Russia

E-mail: tupang@yandex.ru

The “Protey” is a web-based Laboratory Information Management System (LIMS), which aim is to manage, to control, to collect and for optimization of postgenomic studies. Most important features of this information system are flexibility and architecture of data storing. The software flexibility allows to create ontology and data storing architecture directed to specific type and method of the study. Also one more dominant difference of the Protey is an ability to make an external processor for any type of data processing in the system. We have demonstrated usefulness of the ‘Protey’ by the example of study of human liver proteom with methastasis of colorectal cancer after chemotherapy. More than 20 samples of human liver were collected, each of those was fractionated on microsomal and cytosolic fraction. Together with cytochromes P450 activities studies for the microsomal fraction, 2DE gels were obtained for the cytosolic fraction. Totally, about 180-360 the spots were detected on the each gel. Finally the spots were matched between gels and identified by peptide fingerprint analysis. All obtained data and protocols for all stages of the studies were stored in “Protey”. Also values of cytochrome P450 activities were compared with found changes in proteomics data.

SESSION IV.

NANOBIOTECHNOLOGIES AND NANOMEDICINE



INVESTIGATION OF CELLULAR MECHANICS BY AFM

Manfred Radmacher

Institut für Biophysik, Universität Bremen

E-mail: mr@biophysik.uni-bremen.de

We have used atomic force microscopy (AFM) to investigate the mechanical properties of living cells with high resolution. In eukaryotic cells the mechanical properties are mainly determined by the actin cytoskeleton. Thus, the AFM will give information on the architecture and re-organisation of this cross-linked, active polymeric network. E.g. in cell division a stiffening of the equatorial region can be found even before the formation of the cleavage furrow is visible in the topography. This experimental evidence helps distinguishing between different models for cytokinesis. In a variant of AFM, where we have positioned an AFM cantilever spring in front of migrating cells, we were able to measure locally the protrusion forces generated by migrated cells. Our findings can be compared with theoretical predictions, e.g. by elastic ratchet models, which relate the force generated with the polymerization of actin filaments at the leading edge of cells.

NANOCHIPS FOR MEDICAL DIAGNOSTICS

Yurii D. Ivanov, Tatyana O. Pleshakova, Pavel A. Frantsuzov, Alexander V. Ivanov, Olga Nikolskaya, Svetlana E. Nikitina, Sergey P. Radko, Svetlana Yu. Rakhmetova, Nikolay V. Bodoev and Alexander I. Archakov.
Institute of Biomedical Chemistry of RAMS. Pogodinskaya 10, 119121 Moscow, Russia
E-mail: Yurii.Ivanov@ibmc.msk.ru

The major problem of the present-day proteomics lies in the lack of a reaction similar to PCR; hence the impossibility of multiplying various protein molecules, which in turn makes it impossible to enhance the concentrations of assayed biological material. Thus, there arises a problem in diagnostics: protein molecules with concentrations below 10^{-12} M cannot be revealed in biological material. Nanotechnological approaches allow such problems to be effectively solved [3]. We adopted AFM-based nanotechnological methods for use in medical diagnostics and the AFM-nanochips for disease diagnostics have been fabricated. These AFM-nanochips allowed us to reveal hepatitis B and C virus particles in patient serum. The AFM nanochips was used for registration of aptamer/gp120 complexes. The optical biosensor technique appears to be one of the most perspective diagnostic tools also. We have created the biosensoric system registration of hepatitis B and C markers from patient serum in real-time without labels. The optical biosensor method was used for revelation of aptamer/gp120 complexes. Based on the CD-ROM biosensor we are developing a new method of registration of protein-protein interactions. We have created a biosensor based on CD that can convert signals about proteins' molecules on the surface of CD into acoustic signal (molecular music). The CD-chip is a CD with an immobilized protein array. With placing of a sample partner protein onto such a biochip, molecular complexes are formed on the disk surface; as a result the number of reading errors is increased. These errors are played back by CD player as an acoustic signal dependence on time and appear as music. The dependence of reading errors on protein concentration for proteins with various mass – P450BM3, myoglobin, BSA, anti-Hbs, P450scc were obtained. We covalently immobilized one of proteins (e.g. adrenodoxin) on CD, creating this way a CD-biochip, and to carry a procedure of fishing of an protein-partner from solution, then the formation of protein-partners complexes on the surface of CD can be registered with recording of corresponding molecular musics. The possibility of nanowire biosensor for registration of cells and viruses is discussed.

This work was supported by RFBR grant 06-04-08057, FASI contract № 02.512.11.2176.

NANOBIOELECTROCHEMICAL METHODS FOR THE INVESTIGATION OF BIOAFFINITY

Victoria V. Shumyantseva, Tatiana V. Bulko, Elena V. Suprun, and Alexander I. Archakov
Institute of Biomedical Chemistry, Russian Academy of Medical Sciences,
Pogodinskaya Street, 10, Moscow 119121, Russia
E.mail: viktorija.shumyantseva@ibmc.msk.ru

An approach based on volt-amperes' characteristics of voltammograms and amperograms for the analysis of substrate specificity of cytochromes P450 was proposed. To obtain nanoelectrode systems, based on gold nanoparticles on the electrode surface we used colloidal Au solution stabilized with lyotropic liquid crystalline phase of membrane-like synthetic surfactant didodecyldimethylammonium bromide DDAB. The electrochemical behavior of nanostructured electrodes with immobilized cytochromes P450 2B4, 1A2, 3A4, 11A1 (P450_{scc}), and 51b1 (sterol 14 α -demethylase, CYP51b1) in the presence of typical substrates and inhibitors of these forms were studied. Based on the results of amperometry, cyclic voltammetry, voltammetric analysis (DPV and SWV) and intermittent pulse amperometry (IPA), it was possible to conduct a search and to study the kinetic parameters of potential substrates and inhibitors of cytochrome P450. The proposed electrochemical approach is a sort of a bio-bar code of cytochrome P450 for the determination of the substrate/inhibitor P450's competence. The method of electro analysis may be applied to creation of multichannel electrochemical plates (chips, panels) with immobilized cytochromes P450. A novel electrochemical method for the detection of bioaffinity interactions based on a gold nanoparticles sensing platform was developed. Voltammetric method for a direct determination of gold nanoparticles based on stripping voltammetry of Au/Au oxides film was studied. The sensor signal was a gold oxides reduction peak current (area) after E = +1.2V, 30s oxidation. The surface characteristics of the composite electrodes were investigated for the detection of myoglobin - antibody or thrombin - aptamer interactions.

This work was supported by the Federal Agency of Science and Innovations, Ministry of Education and Science of the Russian Federation (State contract № 02.512.11.2105) and by the Interdepartmental Program "Proteomics in Medicine and Biotechnology".

ANALYTICAL NANOTECHNOLOGY ON THE WAY TO REVERSE AVOGADRO NUMBER

Alexander I. Archakov, Yury D. Ivanov
Institute of Biomedical Chemistry RAMS, Pogodinskaya 10 str., Moscow, Russia
E-mail: archak@ibmc.msk.ru

A certain concentration barrier exists in proteomics: the protein molecules residing in the concentration range below 10^{-12} M cannot be identified by trivial proteomic technologies. The atomic force microscopy (AFM) nanotechnological method appears to be one of the most perspective diagnostic tools and enables not to measure protein concentration but merely to count single protein molecules and their complexes. Biospecific fishing method enables to extend the concentration limits of proteomics, as it enables to fetch the protein from the solution and therefore to increase its concentration on the surface of the biochip with immobilized partner proteins. Based on combination of AFM and biospecific fishing method we have created the AFM biochips for registration of hepatitis B and C antigens and ovarian CA125 and prostate cancer PCA biomarkers. The described approach attains the concentrations of about 10^{-15} M; however, to reveal the lower concentrations (e.g. 10^{-18} M) it is necessary to convert the reversible complex antibody (aptamer)/antigen formation reaction into irreversible one. We demonstrated the approach for reveal the HCVcore antigen at 10^{-17} M.

Theoretical calculations show that the combination of these two technologies allows to create new proteomic technologies having detection limit being close to reverse Avogadro number ($10^{-20} - 10^{-21}$ M). This makes possible to overcome the concentration detection limit existing in Proteomics what enables proteomic technologies to equalize with genomic where due to polymerase chain reaction no detection limit exists.

NANOPHARMACEUTICALS: TODAY AND TOMORROW

Olga M.Ipatova, Natalia V.Medvedeva, Oksana Strekalova, Vladimir Prozorovsky,
Alexander I.Archakov
Institute of Biomedical Chemistry RAMS, Pogodinskaya 10 str., Moscow, Russia
Email: inst@ibmh.msk.su

As a matter of fact, all aspects of such developing area as nanomedicine involve application of the achievements of nanotechnologies and nanomaterials to medicine. One can subdivide nanomedicine into 3 conventional units: 1- nanodiagnosticums; 2-nanopharmaceuticals and 3-nanorobots.

Today the development of a new generation of pharmaceuticals -nanopharmaceuticals provides for the usage of new nanomaterials, not existing before, and application of new technologies allowing to produce new pharmaceutical dosage forms from known substances. These new formulations are to be distinguished by high efficiency, bioavailability, absence or decrease of side-effects. For example, fullerenes, dendrimers and nanotubes are related to new nanomaterials intensively used in the development of nanopharmaceuticals. The production of nanocrystals and lipoparticals is considered to be the new processing techniques of nanopharmaceuticals.

The medical product of new generation "Phosphogliv" is developed and applied into medical practice at the State Establishment V.N. Orekhovich Research Institute of Biomedical Chemistry RAMS. The injection form of this preparation represents phospholipid nanoparticles 30-50 nm in diameter. The product is intended for treatment of liver diseases, including acute viral hepatitis. Moreover, a new and unique technology of production of phospholipid nanosystem with particles' diameter 15-30nm, stable at storage has been developed at the Institute. This system is applied for passive and oriented transportation of different medicinal preparations, first of all cytostatics and for increasing their bioavailability and therapeutic efficiency. A new technology of integration into this nanosystem of medicinal substances of different therapeutic types has been created. It was demonstrated that the medicinal preparations in form of phospholipid nanoparticles are 2-4 times more effective than common forms.

Thus, the application of new technological approaches and new nanomaterials offers the challenge in creation of new medicines and systems of their directed transport in the organism.

MEMBRANE NANO-TUBES IN LIVING CELL AND MECHANISM OF THEIR FORMATION

Elena Kiseleva¹, Ksenia N. Morozova¹, Martin W. Goldberg²

¹Institute of Cytology and Genetics, Novosibirsk, Russia

²Durham State University, Durham, UK

E-mail: elenakiseleva@mninangs.ru

Transmission and a high resolution scanning electron microscopy are powerful tools for visualization of nano-structures in living cell. Unusual membrane nano-tubes formed by nerve cell as well as reticulon 4 involved in shaping of endoplasmic reticulum (ER) into tubules and stabilized membrane curvature in different cells have been recently described (Voeltz et al., 2006. *Cell*, 124:573-86). Function of nano-tubes function is an intriguing new goal for modern cellular nano-biology. We investigated the functional role of ER in the nuclear envelope assembly in growing *Xenopus* oocytes by electron microscopy. We found that ER can form 15nm tubules on the nuclear surface. 20nm tubular junctions between fusing ER vesicles and between ER vesicles fusing with the outer nuclear membrane were also observed. Using immuno-electron microscopy we demonstrated that Rtn4 is localised all over the membrane in free ER vesicles and moves to highly curved regions if such vesicles fuse with the outer nuclear membrane. Role of reticulon in nano-tubules formation and nuclear envelope assembly was supported by *in vitro* experiments (Kiseleva et al., 2007. *J. Struct. Biol.*, 160:224-35). According to our model the ER vesicles and nano-tubules fuse with the outer nuclear membrane and move partly through the nuclear pore membrane to the inner nuclear membrane during nuclear envelope assembly. Because of nuclear pore small (~120nm) size the transporting membrane should be strongly curved in pore region. This provides also the effective incorporation of specific proteins for assembling nuclear pores. Formation of nano-tubules with strong membrane curvature stabilized by Rtn4, provides membrane fusion and regulates membrane traffic at nuclear pore during nuclear envelope assembly in non-dividing nuclei.

Work was supported by grant RFBR.

NEW STRATEGY FOR THE DESIGN OF CYTOSOLIC DELIVERY SYSTEMS BASED ON STICHOLYSINS, PORE-FORMING PROTEINS, ENCAPSULATED INTO LIPOSOMES

Maria C. Luzardo¹; Oraly Sanchez¹; Rady Laborde¹; Ali Lopez¹, Lesly Calderon¹, Circe Mesa², Luiz E. Fernandez², Aisel Valle¹, Aracely Lypez¹, Fabiola Pazos¹, Mayra Tejuca¹, Carlos Alvarez¹, Maria E. Lanio¹.

¹Centro de Estudio de Proteínas, Facultad de Biología, Universidad de La Habana.

²Centro de Inmunología Molecular. Cuba.

E-mail: mlanio@fbio.uh.cu

Bacterial pore-forming proteins have been used to design cytosolic delivery systems of macromolecules, such as antigens. Different strategies have been used including the co-encapsulation of cytolytic toxins or peptides with antigen into liposomes in order to improve the antigen-specific cytotoxic T CD8+ lymphocyte response. Sticholysins, (Sts: St I and St II) are 20 kDa cysteineless isotoxins from the sea anemone *Stichodactyla helianthus* forming tetrameric pores in membranes with a 2 nm diameter. Considering that Sts share functional homology with bacterial pore-forming toxins, it was assumed that they could also exhibit the ability to modulate the antigen-specific immune response. The cytotoxic T lymphocyte assays in mice demonstrated the capability of vesicles carrying St I or St II together with ovoalbumin, as model antigen, to promote activation of the T CD8+ lymphocytes mediated response *in vivo*, in comparison with liposomes without St or the positive inducer (P I+C). St I W111C, a mutant forming a reversible inactive dimer stabilized by a disulphide bridge, also induced a similar antigen specific T CD8+ lymphocytes mediated response to St I when encapsulated into liposomes with the antigen. These results show for the first time the immunomodulator properties of Sts, probably as a result of their membranotropic properties.

QUEST FOR UNIQUE BIOMARKERS IN HUMAN FOLLICULAR FLUID

K.Jarkovska, P. Halada¹, H. Skalnikova, J. Martinkova, M. Zilvarova, S. J. Gadher²,
H. Kovarova

Institute of Animal Physiology and Genetics AS CR, v.v.i., Libechov, Czech Republic

¹Institute of Microbiology AS CR, v.v.i., Prague, Czech Republic

²Beckman Coulter International S.A., Nyon, Switzerland

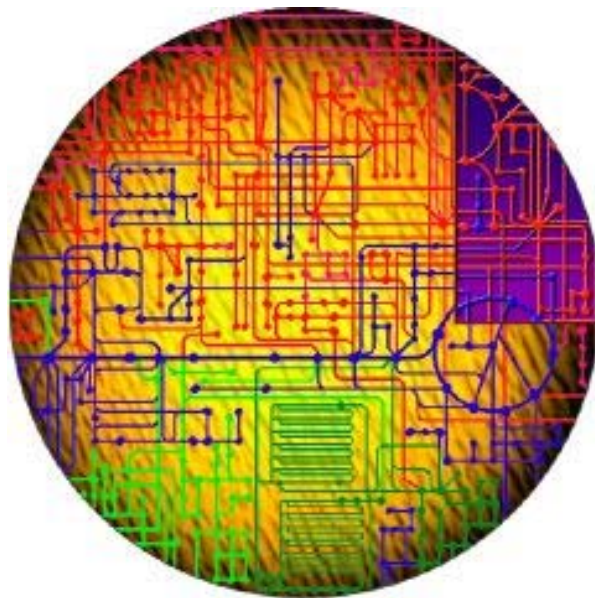
E-mail: kovarova@iapg.cas.cz

Human follicle consists of granulosa cells, oocyte and human follicular fluid (HFF). HFF represents *in vivo* environment of the oocyte and contains a number of biologically active proteins affecting the follicular growth and oocyte maturation before its fertilisation. It is believed that among the proteins in HFF are hidden potential markers indicating future embryonal development. The study was aimed to uncover proteins typical for HFF. We compared 2-D protein maps of HFF vs. serum without/with depletion of 12 highly abundant proteins using combination of ProteomeLab™ IgY-12 Proteome Partitioning Solutions and PF 2D separation system (Beckman Coulter). The proteins characteristic for HFF were selected based on the criterion of their significantly higher level in HFF compared to serum/plasma and were identified by MALDI-MS. Among them, clusterin, which is suggested to interact with components of the complement system and inhibit its activity appears to be potential candidate marker. High expression of clusterin in HFF may be involved in protection of follicular environment from complement mediated damage during fertilization.

The work was supported by grants IQS500450568 (GA AV) and by Institutional Research Concepts AV0Z50200510 (IMIC) and AV0Z50450515 (IAPG).

SESSION V.

SYSTEMS BIOLOGY



NETWORK BIOLOGY OF CELL DIFFERENTIATION

Alexander Kel

BIOBASE GmbH, Halchtersche Str. 33, D-38304 Wolfenbüttel, Germany

E-mail: alexander.kel@biobase-internacional.com

Regulation of cell differentiation is mainly accomplished through executing a program of dynamically changing control of gene transcription. External signals, mediated by cytokines, growth hormones, ions, exert the control on this program by differentially regulating transcription of genes. Such regulation of gene transcription is accomplished through a complex intracellular **signal transduction network** which enables transmission of the signal into the nucleolus of the cells and leads to activation or inhibition of transcription factors (TF). Activated **transcription factors (TFs)** bind to distinct regions of DNA (TF binding sites, TFBS), and, after anchoring at these sites, transmit the regulatory signal to the basal transcription complex that leads to activation or repression of transcription of corresponding genes. Some of TFs are specific for a particular tissue, a definite stage of development, or a given extracellular signal, but most transcription factors are involved in gene regulation under a rather wide spectrum of cellular conditions. It is clear by now that combinations of transcription factors rather than single factors drive gene transcription and define its specificity. Dynamic function-specific complexes of many different transcription factors, so called enhanceosomes are formed at gene promoters and enhancers controlling gene expression in a specific manner. At the level of DNA, the blueprints for assembling such variable TF complexes on promoter regions may be observed as specific combinations of TFBS located in close proximity to each other. We call such structures "Composite Modules (CMs)". Current studies of the molecular mechanisms of complex cellular processes such as cell differentiation boil down to analysis of complex networks of protein-protein and protein-DNA interactions which combines signal transduction and transcription factor networks. We call this field of study – **Network biology**.

We developed a novel computational tool, ExPlain™ for causal interpretation of gene expression data. It performs a rather unusual way of analysis through considering the earlier causes that have led to the observed gene expression changes rather than analysing the later effects of those changes. First of all, promoters of differentially expressed genes are analyzed and specific combinations of transcription factors (Composite Modules) regulating these genes are hypothesized. Next, analysis of signal transduction network upstream of these transcription factors allows us to reveal key signaling molecules that can master the observed gene expression profile. The method utilizes data from three databases (TRANSFAC®, TRANSPATH® and HumanPSD <http://www.biobase-international.com/>). Affymetrix microarray data have been taken from time series study of retinoic acid induced differentiation of promyelocytes to neutrophils. The transcriptomics data were supplemented by proteomics data on the same system. Sets of promoters of differentially expressed genes on different time points have been compared to promoters of genes that did not showed any significant change of expression. Analysis of composite modules has revealed a highly significant combination of transcription factor binding sites for such factors as Egr-2, Myc, SOX-6, IRF-1 and PAX-3 and factor pairs: GATA - Egr and IRF - C/EBP. This composite promoter model was able to discriminate more than 80% of the differentially expressed promoters from the background promoters. Finally, the analysis of the signal transduction pathways upstream of these transcription factors helps to identify several potential key molecules such as calcineurin and PKAc. This analysis helps to build a network model of neutrophils differentiation enabling generation of hypothesis on dynamic mechanisms of cell differentiation.

Parts of the work were funded by EU grants "TRANSISTOR" (MRTN-CT-2004-512285), "EuroDia" (LSHM-CT-2006-518153), VALAPODYN (LSHG-CT-2006-037277) and Net2Drug (LSHB-CT-2007-037590).

1. Kel,A, Konovalova,T, Waleev,T, Cheremushkin,E, Kel-Margoulis,O, Wingender,E. (2006) Composite Module Analyst: a fitness-based tool for identification of transcription factor binding site combinations. *Bioinformatics*. 22, 1190-1197 (2006).

GENE EXPRESSION AND PROTEOMIC PROFILING OF INDUCED HEPATOTOXICITY IN MICE

Victor G. Zgoda, Olga V. Tikhonova, Artur T. Kopylov, Leonid K. Kurbatov and Alexander I. Archakov
V.N.Orekhovich Institute of Biomedical Chemistry RAMS, Pogodinskaya str. 10,
Moscow, 119121, Russia
E-mail: vic@ibmh.msk.su

Methods of gene expression and proteomics profiling by 2DE and 2D LC-MS/MS were applied to study induced hepatotoxicity. For this purpose we used two well known P450 inducers phenobarbital (PB) and 3-methylcholantrene (3-MC) in mice model. After inducers introduction to mice, livers were isolated and cell homogenates were subfractionated by differential ultracentrifugation into cytosol and microsomes.

Both fractions were subjected to 2-DE and 2D-LC MS/MS to generate the proteomic maps of these subcellular fractions. 2-DE yielded 1100 and 800 protein spots for microsomes and cytosol, respectively. In case of 2D-LC MS/MS over 2100 proteins were identified in microsomes and 2400 proteins were identified in cytosolic fraction.

General trends of the fraction-specific alterations and gene expression profiling after 3-MC or PB treatment were evaluated using the Student's t-test and the principal component analysis (PCA). Together with expectable results on P450 and other drug metabolizing enzymes overexpressions we found the significant increasing of expression levels for 30 other proteins. Among them, the highest elevation of expression level was observed for MUPs (over 6- and 4-fold for PB and 3-MC microsomal samples, respectively). The mechanism and the significance of MUPs up-regulation are obscure. It is known though that these proteins bind pheromones and assist in their excretion. Probably MUP participate in the binding of PB and 3-MC or their oxidation products and excretion of the corresponding complexes as well.

Results of molecular dynamics and molecular docking performed on MUP and inducer metabolites confirm our hypothesis on role of MUP in drug excretion.

LATEST ADVANCEMENTS IN HPLC-CHIP/MS WITH APPLICATIONS TO PROTEOMICS AND GLYCOMICS RESEARCH

Rudolf Grimm

Life Science Solutions Unit, Agilent Technologies Inc., 5301 Stevens Creek Blvd.,
Santa Clara, CA 95052, USA

E-mail: rudolf_grimm@agilent.com

Recently a new fully automated and integrated analytical system consisting of a chip-based chromatography system in conjunction with ion trap, time-of-flight, triple quadrupole and quadrupole time-of-flight mass spectrometers was introduced. The microfluidic HPLC-chips are made of laser ablated and laminated biocompatible polyimide films. Sample enrichment, separation and nanoelectrospray tips are fully integrated in the chip device. Chips with different functionality can be easily designed and developed for specific LC/MS applications. The system represents a breakthrough in nanoelectrospray MS sensitivity, chromatographic separation, reproducibility, sample throughput and ease of use.

In this presentation an update about the latest technical developments of the HPLC-Chip/MS system will be provided. Applications of the HPLC-Chip/MS system to the study of complex proteomics samples at the protein and peptide level will be discussed. In addition, data on PTM analysis such as phosphorylation using Chip-ETD ion trap mass spectrometry or oligosaccharide analysis (N- and O-glycans as well as free glycans) using Chip-TOF and Chip-QQQ including glycan MRM measurements in negative ion mode will be presented.

BIOLOGICAL ROBUSTNESS AND THE ENVIRONMENTAL ORIGINS OF 'COMPLEX' HUMAN DISEASES

David M. Balshaw

National Institute of Environmental Health Sciences, US National Institutes of Health
(NIEHS/NIH), Research Triangle Park, NC, USA, 27709

E-mail: Balshaw@NIH.GOV

Modern systems biology represents an approach to integrating across scales of time, space and state to understand the complexity of biological function. This goal is not new but has been enabled by the emergence of high data content techniques (transcriptomics, proteomics, and metabolomics), continually increasing computational power, and the availability of informatics tools for data integration and the visualization of high-dimensional data. As a result it is becoming increasingly evident that biological functions are not ultimately controlled by the reductionist signaling pathways that have been the subject of intense investigation throughout modern biomedical research but by interconnected and dynamic networks of these pathways. These networks enable fine tuning of biological function such that biological systems can respond rapidly and appropriately to certain stimuli while resisting the deleterious effects from harmful perturbations including exposure to environmental stressors. Robustness, the ability of organisms to maintain appropriate function in response to both internal and external stimuli, results from the interconnectedness of these biological networks. Environmentally-induced disease, therefore, results when the damage to the network overcomes the robustness of the system. Knowledge of the networks underlying biological function will provide an understanding of how multiple toxicants interact at different points in common pathways to lead to a single disease outcome; conversely, it will provide insight into how a single toxicant leads to multiple diseases. A third related focus is how induction of common pathways leads to disparate disease outcomes. This understanding of environmental origins of disease processes will lay a foundation for assessing the risks of environmental exposures; in particular the continual challenge of understanding the biological response to 'real world' environmental exposures such as chemical mixtures and chronic, low-level exposures.

METHODS OF THE PREPARATION OF CELLS FOR THE SYSTEMS BIOLOGY STUDIES

Konstantin N. Yarygin^{1,2}, Alexei Yu. Lupatov^{1,2}, Ivan B. Cheglakov¹, Leonid K. Kurbatov¹

¹Institute of Biomedical Chemistry of the Russian Academy of Medical Science, 10 Pogodinskaya Str., 119121 Moscow, Russia; ²Russian State Medical University, 1 Ostrovityanova Str., 117997 Moscow, Russia

E-mail: kyarygin@yandex.ru

At present there are probably two most important research areas where systems biology (SB) methods should be and in fact are being applied to eucariotic cells – carcinogenesis and differentiation/dedifferentiation. The former involves experiments with tumor cells and tumor cell precursors, the latter – experiments with stem, transient amplifying and differentiated cells. Ideally, a cell population used in SB investigations should be live, uniform, relatively cheap to maintain, stable, and easily transferable from one stable state to another. In some cases fixed cells can be used. Within the SB studies program initiated in the Institute of Biomedical Chemistry the laboratory of cell biology concentrates on the preparation of suitable cell material. We created a choice of objects for SB research including a bank of transformed cell lines, two immortal human cell lines transfected with TERT gene, several primary tumor cultures derived from colorectal and renal carcinomas, cultures of mesenchymal stem cells isolated from a number of sources. To separate more homogenous subpopulations of cells we are using cell sorting on FACSAria cell sorter equipped with three different lasers including optional 407 laser and positive and negative selection with immunomagnetic beads labeled with specific antibodies. This provides a tool to isolate stem cells and cancer stem cells based on the exclusion of the Hoechst 33342 dye, and different cell types expressing specific surface markers. We are also using laser capture microdissection to isolate components of solid tumors - cancer stem cells, other cancer cells, tumor stroma blood vessels and invading lymphocytes.

A SYSTEMS BIOLOGY STRATEGY FOR THE STUDIES OF THE UBIQUITIN SYSTEM

Murat R.Gainullin¹, Alexander L.Chernorudskiy¹, Eugene V.Eremin², Maxim E.Astashev³,
Anastasia S.Zhabereva¹, Alejandro Garcia¹

¹Nizhny Novgorod State Medical Academy,

²Institute of Applied Physics RAS, Nizhny Novgorod, Russia

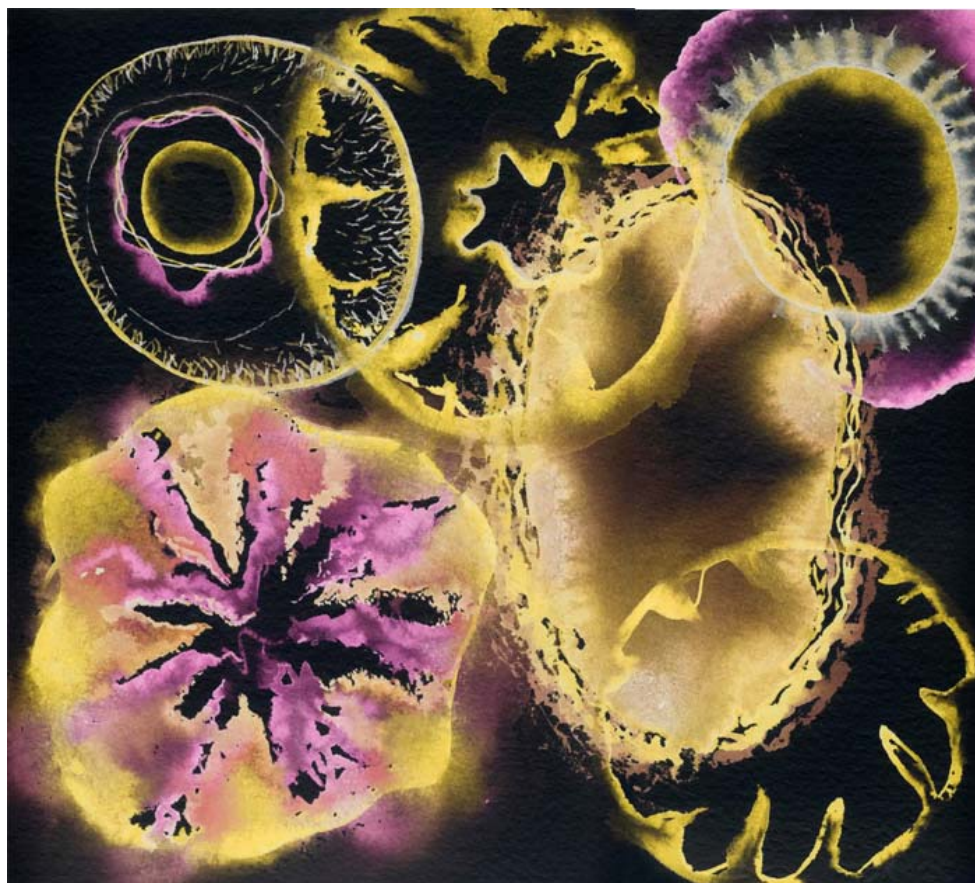
³Institute of Cell Biophysics RAS, Pushchino, Russia

E-mail: biochem@gma.nnov.ru

Protein ubiquitylation is of especial interest due to its role in regulation of diverse cellular processes and involvement in pathogenesis of severe human diseases. The ubiquitin system consists of large number of dynamically interacting proteins and includes: (1) Enzymes catalyzing covalent attachment of ubiquitin to a target protein. (2) Enzymes cleaving a covalent bond between ubiquitin and target proteins. (3) Proteins serving as substrates for modification with ubiquitin. (4) Various “ubiquitin receptors”, recognizing attached ubiquitin(s) as a definite signal and in such a way bridging the ubiquitylation and subsequent ubiquitin-dependent cellular events. In the light of this complexity the ubiquitin system is ideally suited for a systems biology analysis. Our present research comprises several aims. To fill a general need for collecting and systematizing experimental data concerning ubiquitylation we have developed UbiProt Database, a knowledgebase of ubiquitylated proteins (<http://ubiprot.org.ru>). The utility of UbiProt is also being extended for usage in proteomics tasks. To facilitate identification of ubiquitylated proteins by mass spectrometry, we have developed UbIdent, a new tool for virtual fragmentation of target proteins (included in UbiProt dataset or supplied by user) and calculation of the precise masses for peptides bearing modification. Currently we are going to represent the whole ubiquitin system in terms of the system biology. Formalized rules have been introduced for description of the ubiquitylation cascade and deubiquitylating enzymes, their interactions, upstream regulatory events and downstream metabolic outcomes. Respective resource termed «Ubiquitomix» is now under development using BioUML workbench. Further investigations are directed towards supplementation of the existing data, acquired from literature and database searches, with experimental results obtained from proteomic studies as well as examination of specific protein-protein interactions. This strategy combining virtual and experimental approaches provides explanatory and predictive insights into the behaviour of the ubiquitin system and its role in cellular signalling.

SESSION VI.

SYNTHETIC BIOLOGY



THE QUEST FOR A MINIMAL CELL: CONSTRUCTING A SYNTHETIC MYCOPLASMA GENITALIUM GENOME

Clyde A. Hutchison III, Daniel G. Gibson, Carol Lartigue, Gwyn Benders, John I. Glass, Hamilton O. Smith, and J. Craig Venter. The J. Craig Venter Institute, 9704 Medical Center Drive, Rockville, MD 20850, USA
E-mail: chutchison@jcvj.org

The bacterium *Mycoplasma genitalium* has the smallest known genome of any cell that has been grown in pure culture. Its circular 580kb genome has 485 protein-coding genes and 43 RNA genes. In its natural habitat most of its genes are essential and it may be close to a minimal cell. In the laboratory, grown in a rich medium, approximately 100 genes appear to be dispensable based on transposon mutagenesis. We suspect that many, but not all, of these genes could be removed without affecting viability. Our approach to obtaining a minimal laboratory strain of *M. genitalium* is to:

- (1) Chemically synthesize the genome from oligonucleotides. We accomplished this by chemically synthesizing the 582,970 bp *Mycoplasma genitalium* JCVI-1.0 genome. It contains all the genes of wild type *M. genitalium* G37 except MG408, which was disrupted by an antibiotic marker to block pathogenicity and to allow for selection. The genome also contains added “watermark” sequences for identification of the genome as synthetic. The genome was constructed from overlapping “cassettes” of 5-7 kb, assembled from chemically synthesized oligonucleotides. These were joined by *in vitro* recombination to produce intermediate assemblies up to about 144 kb (“1/4 genome”), which were all cloned as bacterial artificial chromosomes (BACs) in *E coli*. We then assembled the four quarters by transformation-associated recombination (TAR) cloning in yeast.
- (2) Construct reduced genomes by eliminating non-essential genes, using a combinatorial library approach.
- (3) Transplant the “minimal” genome library into suitable recipient mycoplasma cytoplasm so that the genomes can “boot-up” and produce a “minimal cell”. We have successfully transplanted naked *M. mycoides* DNA genomes into *M. capricolum* recipient cells, and we are experimenting with similar methods to transplant *M. genitalium* genomes into suitable recipient mycoplasma cells. The final step will be to screen for viable cells with the smallest genomes.

SYNTHETIC PEPTIDE ARRAYS IN SEARCH FOR DIAGNOSTIC ANTIBODIES AND VACCINE DEVELOPMENT

Ekaterina F. Kolesanova, Tatiana I. Kuzmina, Maxim A. Sanzhakov, Tatiana V. Abramihina, Elena Yu. Aleshina, Tatiana E. Farafonova, Alexander I. Archakov, Irina N. Khropova*, Svyatoslav G. Cheshik*

Institute of Biomedical Chemistry, Russian Academy of Medical Science, Pogodinskaya, 10, Moscow 119121, Russia

*D.I. Ivanovsky Institute of Virology, Russian Academy of Medical Science, Moscow
E-mail: EKolesanova@yandex.ru

Peptide arrays comprise convenient tools for protein-protein interaction site mapping, including epitope mapping. Peptide arrays composed of overlapping peptide fragments that cover highly conserved sites of hepatitis C virus (HCV) envelope proteins as well as different genetic variants of the hypervariable region 1 (HVR1) of HCV envelope protein E2 were prepared by means of the improved schedule of multiple parallel peptide synthesis on derivatized polyethylene pins. The synthesis schedule improvement was achieved by the use of a more efficient reagent for 9-fluorenyl(oxycarbonyl) alpha-amino-group deprotection and thorough choice of the amino acid coupling step conditions. Peptides were biotinylated for the further attachment to the surface of ELISA plates covered with streptavidin. These peptide arrays were used for B-epitope mapping of HCV envelope protein E2 using anti-(whole protein) antibodies from rabbit antisera and for the search of peptide-specific antibodies in sera of HCV-infected patients. Two novel antigenic determinants were revealed in the conserved region CR2 of E2 protein. A wide cross-reactivity was observed between differing genetic variants of HVR1 N-terminal octapeptide fragment pointing out to the possibility of the use of this site for immunodiagnostic purposes and vaccine development despite of its variability. The ability of anti-peptide antibodies to detect picomolar peptide concentrations indicated the putative applicability of these antibodies for detecting the HCV infectious material in biological samples. Peptide arrays were also successfully used for assessing the immune response to synthetic peptide vaccine constructs.

The work was supported by RFBR grants 03-06-33033, 04-07-01080, 04-07-12117, and the RAMS Program "Proteomics for Medicine and Biotechnology".

STICHOLYSIN II STRUCTURE AND FUNCTION CAN BE MODELLED BY SYNTHETIC PEPTIDES REPRODUCING THE N-TERMINUS OF THIS PORE-FORMING PROTEIN

Uris Ros¹, Maite Lopez¹, Joana Paulino², Edson J. Crusca², Mayra Tejuca¹, Fabiola Pazos¹,
Diana Martinez¹, Eduardo M. Cilli³, Tirso Pons¹, Maria E. Lanio¹,
Shirley Schreier², Carlos Alvarez¹

Center for Protein Studies, University of Havana, Cuba

²University of Sro Paulo, Brazil

³State University of Sro Paulo, Araraquara, Brazil

E-mail: calvarez@fbio.uh.cu

The pore-forming protein sticholysin II (StII) can be used to build immunotoxins against tumoral cells and systems for delivering molecules to cell cytosol. The 3D structure of StII is comprised of a β -sandwich core flanked on the opposite sides by two α -helices, one of them located at the N-terminus. The first thirty N-terminal residues are the best candidate for pore formation being the only portion of the molecule that can change conformation without perturbing the protein fold. In order to clarify whether the N-terminus may mimic the function of StII, we have studied the dynamic, conformational and functional properties of synthetic peptides containing residues 1-30 (StII₁₋₃₀ ALAGTIIAGASLTFQVLDKVLEELGKVS RK) and 11-30 (StII₁₁₋₃₀). StII₁₋₃₀ was more active than StII₁₁₋₃₀ in lipid monolayers and vesicles, and erythrocytes forming pores of around 1nm radius. UV-CD studies showed that StII₁₁₋₃₀ displayed a random conformation while StII₁₋₃₀ underwent aggregation with increasing concentration, pH, and ionic strength. In the presence of trifluoroethanol and upon binding to detergent micelles, StII₁₋₃₀ showed a higher propensity to acquire α -helix. Peptide MD simulations (10 ns, Gromacs) in explicit water corroborated some of the experimental data since StII₁₋₃₀ adopts a β -sheet structure while StII₁₁₋₃₀ a helical conformation indicating that the sequence 1-10 is determinant in peptide folding. Taken together, these results demonstrate that synthetic peptides can mimic conformational and functional characteristics of St II N-terminus, emphasizing the contribution of the sequence 1-10 to folding, binding and pore formation.

PEPTIDES INHIBITOR OF THE BINDING OF ANTI-TPO AUTOANTIBODY: COMPUTER DESIGN, SYNTHESIS AND *IN VITRO* INVESTIGATIONS

Irina V. Shutova, Olga V. Gribovskaja, Olesja V. Tsyganova, Vladimir P. Golubovich
The Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus,
Minsk, Belarus

E-mail: shutova@iboch.bas-net.by

Autoimmune thyroid diseases are developed as the result of the immune system response to natural proteins of the thyroid – thyroglobulin, receptor of thyrotrophic hormone and thyroid peroxidase. At that high level of the specific autoantibodies to thyroid antigens are observed in serum of the patients suffering from these diseases. Defining and studying thyroid antigen's determinants will allow developing new methods of autoimmune thyroid diseases treatment and diagnostic. Our objectives were (i) the computer designing new peptides epitopes of the thyroid peroxidase, (ii) synthesis of these compounds and (iii) *in vitro* investigations.

Human thyroid peroxidase (hTPO) is protein with indeterminate 3D structure that has high homology with human myeloperoxidase (hMPO) primary structure. Therefore, at first we are aligned the primary structure of hTPO and hMPO proteins. Then with helping program complex designed by authors, we are defined amino acid residues Glu⁷³⁴Gln⁷³⁸Asp⁷³⁹Lys⁷⁴¹ that are closely spaced on the surface of hTPO and formed potential epitope of this protein. Based on this we are designed, synthesized and *in vitro* investigated several peptides.

Synthesis peptides carried out by methods classical peptide of chemistry in a solution by sequential addition tert-butylloxycarbonyl-(Boc-)-amino acids to C-end fragments with use dicyclohexylcarbodiimide (DCC) as the condensing agent and hydroxybenzotriazole (HOBT) antiracemic addition. The purity and structure of peptides are confirmed by methods thin-layer chromatography, HPLC, FAB-mass-spectrometry, amino acid hydrolysis.

In model ELISA system it was shown that one of peptide strongly inhibited binding of special anti-TPO autoantibody with immobilized hTPO. The rate of inhibition was in the limit 80% when used concentration of peptide equal 1mM.

High activity of this peptide is allowed to use it in development diagnostic systems and therapeutic agent able to modulate immune responses.

SESSION VII.

ISTC

I S T C



М Н Т Ц

A NEW BACTERIAL L-ASPARAGINASE FOR LEUKEMIA TREATMENT

Nikolai N. Sokolov, Anastasia V. Kuchumova, Yulia A. Leonova, Yurii V. Gervaziev, and
Julya Krasotkina.

Institute of Biomedical Chemistry RAMS, Moscow

E-mail: biomed_2005@bk.ru

Bacterial L-asparaginase is widely employed in the treatment of acute lymphoblastic leukemia. This enzyme is also a component of the chemotherapy of acute and chronic myelogenous leukemia. Recently the attempts to use bacterial L-asparaginases in the treatment of Hodgkin and non-Hodgkin diseases, myelosarcoma, multiple myeloma, and AIDS-related lymphomas have been reported. Yet, clinical application of L-asparaginase is always limited because of L-glutaminase activity endowed by the enzyme. Serious liver disorders, acute pancreatitis, hyperglycemia, immunosuppression and other dysfunctions are the results of glutamine deprivation. The search for an optimal therapeutic enzyme started more than 30 years and still has not resulted in any notable success. Nowadays, bioinformatics methods provide a powerful tool to search a target enzyme with desired properties for further cloning. In our work we have searched the currently available nucleotide and protein databases and asparaginase from *Pectobacterium atrorepticum* (PDB entry [CAG75243](#)) has been chosen. This enzyme has been cloned, expressed in *Escherichia coli* BL21(DE3) and characterized. Kinetic properties of *Pectobacterium atrorepticum* recombinant asparaginase has been determined towards two physiological substrates L-asparagine and L-glutamine. PaA catalytic parameters K_{cat} (828 s^{-1}) and K_m (92 mM) for L-asparagine were comparable to the same constants of *Erw.chrysanthemi* asparaginase that currently used in clinical practice for leukemia treatment. Glutaminase activity of PaA did not exceed 2% from asparaginase activity under substrate saturating conditions. Antiproliferative experiments showed that *in vitro* PaA suppresses the growth of human cervical cancer (HeLa), chronic myeloid leukemia (K 562) cells and less effectively breast cancer (MCF-7) cells. The reported results indicate *Pectobacterium atrorepticum* asparaginase to be very promising for therapeutic application in cancer treatment.

The work is fulfilled at financial support of the ISTC (#2828) and grants RFBR #06-04-49792-a.

UNSTABLE CHROMOSOMAL REGIONS IN THE HUMAN GENOME: COLON CANCER IMPLICATIONS

Nina J. Oparina^{1*}, Georgy S. Krasnov¹, Vladlen S. Skvortsov², Tamara D. Mashkova¹

¹Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov street, 32, Moscow, Russia

²Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Pogodinskaya street, 10, Moscow, Russia

E-mail: oparina@gmail.com

Human genome is enriched with non-coding regions, composed from not only from regulatory DNA, but also from different kinds of repetitive DNA. So-called ‘segmental duplications’ or ‘low-copy repeats’ contain large (up to 100 kb and more) segments, highly homologous to other chromosomal regions. It was recently demonstrated that segmental duplications flanking genomic ‘hot spots’ could be involved in the homologous recombination leading to chromosomal disorders, including the chromosomal instability in cancer cells. Frequency of segmental duplications varies a lot between different genomic locations. Unfortunately, the ‘whole-genome’ assembly includes only euchromatic part of the genome and duplications-enriched heterochromatin regions are absent. We have developed an approach of mapping the duplication-prone and duplication-proof regions using the human genome shotgun data. Our method let us determine regions with the lowest and the highest frequencies of segmental duplications. To check the hypothesis that the fate of these two types of genomic loci differs in cancer, we have compared the transcriptomics profile of genes located in some of these regions in colorectal cancer. Colorectal adenocarcinoma is one of the the most severe and common cancers. We have analyzed the set of well-annotated EST (“expressed sequence tag”) libraries from from normal colorectal mucosa, nonmetastatic adenocarcinoma and the pool of ESTs from colorectal cancer cell lines and metastatic tissues. Housekeeping genes showing the same level of transcription in all normal tissues differs in their cancer-associated expression changes. We have demonstrated that the housekeeping genes located in duplications-prone regions showed much more cancer-related variations in colorectal transcriptome that analogous gene located in duplication-proof regions. Also we have shown the higher frequency of CpG-related mutations in EST sequences of genes located in duplications-prone regions.

THE MODEL OF TOXIC ACTION OF PROGESTERONE-LIKE CHEMICALS

Vladlen S. Skvortsov

Institute of Biomedical Chemistry RAMS
10, Pogodinskaya str., Moscow, 119121, Russia
E-mail: vladlen@ibmh.msk.su

This work analyzes the toxic action of chemicals due to their interaction with nuclear receptors. Prediction of side and toxic effects becomes more and more important in development of new medicines and application of known drugs. But the experimental study is time-consuming and highly expensive. In other hand in most cases the toxicity has the complex and multifactoral nature and cannot be described by simple QSAR equation. The effectors of nuclear receptors are one of such cases. One of the main features of these receptors is very high structure similarity of ligand binding domain (LBA). It is naturally to guess that there are a lot of nonspecific interactions between effectors and wrong receptor. Some of them are known but most are not. Suggested method based on multiple docking procedures: one chemical to different receptors. All chemicals were molecules similar to progesterone or derivatives progesterone. Finally we have obtained the set of more than 100 substances that bind to 11 different LBA. The estimation of interaction characteristics was done by MMPBSA method and the model for toxicity prediction was constructed on the basis of neural network.

This work was carried out at support of ISTC grant 3777.

COMPUTER-AIDED DISCOVERY OF NEW HIV-1 INHIBITORS

Vladimir Poroikov¹, Dmitry Druzhilovsky¹, Svyatoslav Shevelev², Marina Gottikh³,
Marc Nicklaus⁴

¹Institute of Biomedical Chemistry of Rus. Acad. Med. Sci., 119121, Moscow, Russia;
²Institute of Organic Chemistry of Rus. Acad. Sci., Moscow, 119991, Russia; ³Institute of
Physical-Chemical Biology, Moscow State University, 119992 Moscow, Russia;

⁴National Institutes of Health, National Cancer Institute, Frederick, MD, 21702, USA;
E-mail: dmitry.druzhilovsky@ibmc.msk.ru

Despite the recent FDA approval of the first HIV-1 integrase inhibitor Raltegravir for clinical use, efforts to find new HIV-1 integrase inhibitors are still necessary due to the emerging resistance to these agents. The purpose of this study was finding of new HIV-1 integrase inhibitors using computer-aided drug design methods, chemical organic synthesis and biological activity testing in *in vitro* experiments.

A database with information about known HIV-1 integrase inhibitors was developed, and our computer system for prediction of HIV-1 inhibitors was re-trained on the new data. Many computer experiments were performed with commercially available compounds' samples databases consisting of millions molecules. over 200 compounds were selected as hits, synthesized or purchased from vendors of commercially available samples, and tested *in vitro* on strand transfer and 3' processing inhibition.

As a result of these studies, over 20 compounds were identified as HIV-1 integrase inhibitors. The most active compounds have IC₅₀ values in the micromolar and sub-micromolar range, placing them below an important threshold because the HIV-1 integrase inhibitors are considered as being active if their IC₅₀<100 μM.

It is important to mention that the discovered compounds belong to the chemical series, in which HIV-1 integrase inhibiting activity had never been found before. Therefore, these compounds can be considered as New Chemical Entities (NCEs).

Further prospects in optimization of structure & properties of the anti-HIV agents will be discussed.

This research was supported by ISTC/BTEP grant # 3197/111.

SESSION VIII.

SCI-MIX



NOVEL STRATEGIES TO INCREASE DEPTH OF ANALYSIS FOR BIOMARKER DISCOVERY USING PROTEOMICS

Sam Hanash

Fred Hutchinson Cancer Research Center

E-mail: shanash@fhcrc.org

Although our understanding of the molecular pathogenesis of common types of cancers has improved considerably, the development of effective strategies for cancer diagnosis and treatment have lagged behind. The vast dynamic range of protein abundance in plasma and the likely occurrence of tumor derived proteins in the lower range of protein abundance represent a major challenge in applying a proteomic based strategy for their identification. A combination of innovative strategies promises to overcome these challenges. Recent experience in comprehensive profiling of plasma proteins indicates that low abundance proteins may be identified and quantified with high confidence following extensive plasma fractionation and with the use of protein tagging procedures and high-resolution mass spectrometry. From an experimental design point of view, most cancer biomarker studies, including those aimed at identifying markers for early detection, are initiated with analysis of specimens from newly diagnosed subjects. The discovered candidate markers are subsequently investigated for their utility for early cancer diagnosis. A preferred approach for discovery of such markers is to utilize plasma obtained at a pre-clinical stage, prior to the diagnosis of cancer. Another implementation of this approach is through the use of mouse models of cancer that potentially represent an efficient means for uncovering diagnostic markers because of the ability to engineer mice that harbor genetic alterations known to be associated with tumors in humans, and because of the limited heterogeneity among mice bred under uniform conditions and the ability to sample blood in a standardized manner, at defined stages of tumor development. Another approach is to identify tumor antigens that elicit an antibody response. Panels of such antigens may have utility in cancer screening, diagnosis or in establishing prognosis. Such antigens may also have utility in immunotherapy against the disease. One approach for comprehensive analysis of proteins in their modified forms is through protein arrays. The current status of these approaches will be presented.

DISTINCT PARTS OF POMC AMINO ACID SEQUENCE IN THE REGULATION OF FAT AND CARBOHYDRATE METABOLISM

Yuri A. Pankov

Institute of Experimental Endocrinology, Endocrine Research Center of Rosmedtecnology,
Moscow, Russian Federation

E-mail: yuri-pankov@mtu-net.ru

Homozygous mutations in *leptin* gene induce obesity and insulin resistance. Proopiomelanocortin (POMC) peptides (MSHs) and their receptor (MC4-R) expressed in hypothalamus are mediators of leptin action on fat metabolism. MC4-R knockout (MC4-RKO) mice (homo- or heterozygote) show obesity, hyperinsulinemia, and hyperleptinemia. In the MC4-RKO mice hyperinsulinemia and impaired insulin tolerance usually preceded the onset of obesity (1). POMC transgene expressed in brain effectively normalized hyperglycemia and insulin resistance, but it only partially reversed obesity and hyperphagia in the leptin-deficient mice (2). In this research, heterozygous mutations in human *pomc* gene had been studied in the obese adults (without T2DM) and in the children with a simple or complicated obesity. PCR, SSCP, and DNA sequencing were applied to search for POMC mutations. The heterozygous insertion of 176RA in combination with E180X near the N-terminal of β -MSH were defined in 8 obese patients with BMI=37.3 \pm 9.2. Among them 5 women had BMI= 43.2 \pm 8.7 and 3 men showed the lower BMI=27.2 \pm 5.3. This mutation partly blocked the expression of β -MSH and β -endorphin, but it could not alter the structures of α - and γ -MSH coded by *pomc* gene. The heterozygous F118L substitution in active core of α -MSH was identified in 3 obese women with BMI=31.1 \pm 4.2 and it was not detected in men. This mutation could disturb the efficiency of α -MSH, but it could not impair the structure and function of β -MSH and β -endorphin. The data presented suggested that β -MSH might be a more essential mediator of leptin action in human than α -MSH. In the children with obesity complicated by hyperinsulinemia the prevalence of heterozygous 73SSG insertion near C-terminal of γ -MSH in 16K peptide of POMC was 3 times higher than it was in the children and adults with the simple obesity. The results obtained suggested that POMC peptides derived of 16K fragment might be essential mediators of hormonal signals activating the efficacy of carbohydrate metabolism regulation and they could facilitate the peripheral tissues sensitivity to insulin. This view complies with the reduced obesity, enhanced peripheral insulin sensitivity and attenuated hyperinsulinemia in the transgene mice overexpressing α - and γ_3 -MSH (but not β -MSH and β -endorphin) (3).

1. Fan W et al., *Endocrinology* 2000; 141: 3072
2. Mizuno TM et al., *Diabetes* 2003; 52: 2675
3. Savontaus E et al., *Endocrinology* 2004; 145: 3881

PROTEOMICS IN BIOLOGICAL PSYCHIATRY

Irina S. Boksha, Gulnur Sh. Burbaeva, Olga K. Savushkina, Elena B. Tereshkina,
Marina S. Turishcheva, Lubov' I. Starodubtseva, Elena A. Vorobyeva
Mental Health Research Center (MHRC), Russian Academy of Medical Sciences,
Moscow, Zagorodnoe Sh., 2-2, Russia.
E-mail: boksha_irina@yahoo.co.uk

Two directions of proteomics/metabolomics application are currently developing in biological psychiatry. The first is the brain proteomics (search for “fingerprint” proteins, amounts of which are changed typically for distinct mental/ neurodegenerative pathology) that is useful for understanding of pathogenesis of the diseases. The second is the search for vital (blood) diagnostic and prognostic “protein markers” of mental disorders. The study in the first direction has been elaborated at MHRC for the past 15 years (immunochemical identification of proteins on protein maps of brain tissue has been employed). Significant quantitative alterations of key enzymes of energy and neurotransmitter metabolism in Alzheimer’s disease and schizophrenia have been demonstrated as a result of the comparative study. The role of energy and glutamate metabolism enzymes in the pathogenesis of these diseases has been elucidated and recognized due to the proteomics/metabolomics approaches. Diversity of isoforms in the key enzymes metabolizing glutamate in human brain has been discovered. The most important finding is that different patterns of correlative links between the amounts of key enzymes and major proteins found in healthy brain and brain of patients with schizophrenia open a new perspective in understanding of impaired regulation of brain enzymes’ levels in mental disorders. The second direction is the search for “clinical – biological” correlations and “protein markers” aiming diagnostic and prognostic value for pathological processes in mental and neurodegenerative diseases, namely, search for blood cell’ proteins, which amount is specifically changed and associated with a certain mental or neurodegenerative disorder, thus enabling very early diagnosis and prognosis. Alterations in protein amounts are monitored in the treatment course of mental disorders. The search for correlation between alterations of protein composition (in the course of treatment) and antipsychotic therapy efficacy is carried out with the aim of “predictor” revealing, enabling to predict individual efficacy of the treatment.

PROTEOMIC IDENTIFICATION OF ISATIN BINDING PROTEINS

Olga A. Buneeva, Victor G. Zgoda, Arthur T. Kopylov, Vladimir F. Pozdnev, Vivette Glover*, and Alexei E. Medvedev

Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, 10 Pogodinskaya street, Moscow, 119121 Russia

*IRDB, Imperial College London, UK

E-mail: olga.buneeva@ibmc.msk.ru

Isatin (indole-2,3-dione) is an endogenous indole which has a distinct and discontinuous distribution in the brain and in other mammalian tissues and body fluids. It exhibits a range of pharmacological and behavioural effects and [³H]isatin binding sites have been already characterized in various brain structures in terms of their K_d and B_{max} (Crumevolle-Arias et al., 2008). Nevertheless, particular biological targets remain poorly characterized. In this study, using N-(6-aminocaproyl)-5-amino isatin as the affinity ligand, and proteomic identification employing reverse-phase nano-LCMS/MS, Spectrum Mill MS Proteomics Workbench Rev A.03.03.078 and comparison of experimental data to the Swiss Prot rat subset database we have indentified individual isatin binding proteins of rat brain. Affinity chromatography of clear Triton X-100 lysates of whole brain homogenates resulted in adsorption of 3.5 ± 0.8% of total protein applied. Subsequent elution of adsorbed proteins by isatin and NaCl followed by their proteomic analysis resulted in identification of 30 individual isatin binding proteins. The identified proteins can be subdivided into the following groups: i) proteins/enzymes involved in energy generation and carbohydrate metabolism (n=11); ii) cytoskeletal proteins; iii) proteins/enzymes involved in regulation of gene expression, protein folding and cell differentiation (n=6); iv) proteins/enzymes involved in cell signaling (n=2); vi) antioxidant enzyme peroxiredoxin 2, also known as thioredoxin-dependent peroxireductase. One of the proteins, “fished” from the whole brain homogenate by means of aminocaproyl-isatin-Sepharose, glyceraldehyde-3-phosphate dehydrogenase, was already known as an isatin binding protein and a specific interaction between isatin and purified glyceraldehyde-3-phosphate dehydrogenase was demonstrated by means of two independent approaches, a biosensor technique (using an isatin analogue immobilized onto an optical biosensor cell) and a radioligand analysis employing [³H]isatin (Medvedev, Buneeva, Gnedenko et al., 2006).

This work was supported by grants from The Wellcome Trust (072381/Z/03/Z) and Russian Foundation for Basic Research (06-04-48355, 07-04-00803).

**GENE EXPRESSION OF MULTIPLE PATHWAYS IN HUMAN BREAST CANCER
AND TUMOR PROGRESSION USING GENOMELAB™ GEXP GENETIC
ANALYSIS SYSTEM.**

Suresh Jivan Gadher

Beckman Coulter International S.A., 22, rue Juste Olivier, Casa Postale 1059,
CH – 1260 Nyon 1,
Switzerland

E-mail: sgadher@beckman.com

The GenomeLab GeXP Human Breast Cancer*Plex* methodology enables researchers to study multiple gene pathways in human breast cancer and tumor progression, including cell cycle regulation, cell growth, differentiation, apoptosis, cancer suppression, hormone response and DNA damage. Many of the genes were selected from publications for their association with clinical outcome of breast cancer patients based on statistically analyzed and similarly clustered gene expression patterns. The link between the genes in the panel and breast cancer provides important insights to disease pathology of samples analyzed. The methodology features primer mixes for 21 commonly studied, breast cancer related genes, plus three reference genes and one internal control. Together, these 25 genes with the GenomeLab GeXP Genetic Analysis System, deliver multiplexed quantitative analysis for hundreds of samples in one day, providing higher throughput than real-time PCR[†], resulting in significant time and cost savings. The GenomeLab GeXP technology was developed to fill the gap between whole genome microarrays and single gene, real-time PCR. Using a combined gene-specific, universal priming strategy, the GeXP technology enables a high level of multiplexing without compromising the detection sensitivity of the process. With this technology, researchers can measure up to 30 genes in a single reaction in each of hundreds of samples using as little as 25 ng total RNA. It is ideal for signature validation, pathway analysis and drug characterization. The GenomeLab GeXP Human Breast Cancer*Plex* Kit streamlines the application of this technology in cancer research.

[†] The PCR process is covered by patents owned by Roche Molecular Systems, Inc.

SENSITIVITY ENHANCEMENT USING GOLD PROBES FOR DETECTION OF THE NUMBER OF TELOMERIC REPEATS

Sergey P. Radko, Svetlana A. Voronina, Alexander V. Gromov, Oksana V. Gnedenko,
Nikolay V. Bodoev, [Alexis S. Ivanov](#), Alexander I. Archakov
Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, Moscow, Russia
E-mail: alexei.ivanov@ibmc.msk.ru

Possibility of direct detection of telomerase activity was studied by using the optical biosensor Biacore 3000 based on the surface plasmon resonance effect. It has been found that a non-specific sorption of cell lysate components on the chip surface masks an elongation of the telomerase primers, therefore hampering quantification of the telomerase activity. Streptavidin-coated gold nanoparticles were used for conjugates with biotinylated oligonucleotide probes, containing the sequence complementary to telomeric repeats. Conjugates of gold nanoparticles with probe cause a higher refractive index and mass change per a binding event than probes alone. As a result, the gold particles can be used to enhance the sensitivity of assays performed on SPR-biosensors. Analysis of interaction of nanoparticle-probe conjugates (GP:CX), streptavidin-probe conjugates (ST:CX) and probe alone (CX) with immobilized oligonucleotide (TR4) containing four telomeric repeats was performed. The rate constant of association of GP:CX/TR4 complex decreases approximately 3-fold compare to that for CX/ TR4 complex. Detected response for GP:CX is increased by about 4- and 70-fold than that for ST:CX and CX, respectively.

The work was supported by RFBR grant 07-04-01605-a.

Author Index

A

Abramihina T.V.....	62
Aleshina E.Yu.....	62
Alexandrov K.E.....	42
Alvarez C.....	51, 63
Archakov A.I.....	14, 15, 17, 21, 29, 36, 38, 46, 47, 48, 49, 55, 62, 76
Astashev M.E.....	59

B

Balshaw D.M.....	57
Benders G.....	61
Beretta L.....	12
Bernhardt R.....	16
Bodoev N.V.....	21, 29, 46, 76
Böhmer S.....	16
Boksha I.S.....	73
Branco R.....	41
Bulko T.V.....	47
Buneeva O.A.....	74
Burbaeva G.Sh.....	73

C

Calderon L.....	51
Carapito C.....	16
Carlson B.A.....	24
Cheglakov I.B.....	28, 58
Chernorudskiy A.L.....	59
Cheshik S.G.....	62
Cho S.....	13
Cilli E.M.....	63
Crusca E.D.....	63

D

Dmitrieva-Zdorova E.V.....	29
Druzhilovsky D.S.....	69

E

Eremin E.V.....	59
Ershov P.V.....	15

F

Farafonova T.E.....	62
Fernandez L.E.....	51
Filimonov D.A.....	37, 40, 42
Filippov I.V.....	39

Frantsuzov P.A.	46
Frenkel F.F.	32

G

Gadher S.J.	19, 52, 75
Gainullin M.R.	59
Garcia A.	59
Generozov E.V.	27
Gervaziev Yu.V.	66
Gibson D.G.	61
Gladyshev V.N.	24, 25
Glass J.I.	61
Gloriozova T.	40
Glover V.	74
Gnedenko O.V.	21, 76
Goldberg M.W.	50
Golovin A.	35
Golubovich V.P.	64
Gottikh M.B.	69
Govorun V.M.	27
Gribovskaja O.V.	64
Grimm R.	56
Gromov A.V.	76

H

Halada P.	18, 19, 52
Hanash S.	71
Hatfield D.L.	24
Henrick K.	35
Hutchison III C.	61
Hwang K.H.	16

I

Ilina E.N.	26
Ipatova O.M.	49
Ivanek R.	34
Ivanov A.S.	15, 21, 76
Ivanov A.V.	46
Ivanov Yu. D.	46
Ivanov Yu.D.	48

J

Jarkovska K.	52
Jeong A.-S.	13

K

Karalkin P.A.	28
Karuzina I.I.	14, 43
Kel A.	54
Kelly S.	23

Khropova I.N.	62
Kim H.	13
Kim K.-Y.	13
Kiseleva E.	50
Klener P.	18
Koborova O.N.	37
Kolesanova E.F.	62
Kononikhin A.S.	20
Koptsov Yu.O.	43
Kopylov A.T.	55, 74
Korotkov E.V.	32
Kostin P.A.	27
Kovarova H.	52
Krasnov G.S.	67
Krasotkina Ju.	66
Kuchumova A.V.	66
Kurbatov L.K.	28, 55, 58
Kuzmina T.I.	62
Kwon M.-S.	13

L

Laborde R.	51
Lagunin A.A.	40
Lagunin A.V.	37
Lanio M.E.	51, 63
Lartigue C.	61
Lee E.-Y.	13
Lee H.	13
Lee H.-J.	13
Leize E.	16
Leonova Yu.A.	66
Lisitsa A.V.	14, 36, 38, 43
Lopez Ali.	51
López Ar.	51
Lopez M.	63
Lupatov A.Yu.	28, 58
Luzardo M.C.	51

M

Martinez D.	63
Martinkova J.	52
Mashkova T.D.	67
Medvedev A.E.	74
Medvedeva N.V.	49
Mesa C.	51
Mezentsev Yu.V.	15
Miroshnichenko Yu.V.	36
Molnar A.A.	15
Morozova K.N.	50
Moshkovskii S.A.	14, 17

N

Na K.....	13
Nicklaus M.C.....	39, 69
Nikitina S.E.	46
Nikolaev E.N.....	20
Nikolskaya O.N.....	46

O

Oparina N.J.....	67
------------------	----

P

Paik Y.-K.....	13
Pajares M.A.	20
Pankov Yu.A.....	72
Paulino J.	63
Pazos F.....	51, 63
Pérez-Sala D.	20
Petrak J.	18, 34, 36
Petushkova N.A.....	14, 43
Pleiss J.	41
Pleshakova T.O.....	46
Pogoda T.V.....	27
Ponomarenko E.A.....	36
Pons T.....	63
Popov I.A.....	20
Poroikov V.V.....	37, 40, 42, 69
Portillo F.....	20
Pozdnev V.F.....	74
Prozorovsky V.....	49
Pyatnitskiy M.A.....	38
Pyatnitsky M.A.....	17

R

Radko S.P.	21, 46, 76
Radmacher M.	45
Rakhmetova S.Yu.....	21, 46
Reytor E.....	20
Ros U.....	63
Rudenko V.....	43

S

Sanchez O.....	51
Sanzhakov M.A.....	62
Savushkina O.K.....	73
Schreier S.....	63
Seifert A.....	41
Serrano H.....	20
Shevelev S.A.....	69
Shumyantseva V.V.....	47
Shutova I.V.....	64

Simonova T.	18
Sirim D.	41
Sitzmann M.	39
Skalnikova H.	19, 52
Skvortsov V.S.	33, 67, 68
Smith H.O.	61
Sobolev B.N.	42
Sokolov N.N.	66
Starodubtseva L.I.	73
Strekalova O.	49
Suprun E.V.	47

T

Tatzel S.	41
Tejuca M.	51, 63
Tereshkina E.B.	73
Thiele H.	31
Tikhonova O.V.	55
Toman O.	18, 34
Toropygin I.Yu.	17
Tsyganova O.V.	64
Turishcheva M.S.	73

V

Valle A.	51
Van Dorsselaer A.	16
Vargas R.	20
Vázquez J.	20
Velasco R.	20
Venter J.C.	61
Veselovsky A.V.	33
Vlasova M.A.	17
Vorobyeva E.A.	73
Voronina S.A.	76
Voronko O.E.	29
Vyoral D.	34

W

Wilzewski B.	16
-------------------	----

X

Xu X.-M.	24
---------------	----

Y

Yarygin K.N.	28, 58
-------------------	--------

Z

Zakharov A.V.	37
Zgoda V.G.	55, 74

Zhabereva A.S.	59
Zilvarova M.	52
Zivny J.	18, 34



Pharmstandard is the leader among domestic manufacturers and the third largest pharmaceutical company operating in Russia overall. Pharmstandard is a leader of the biggest commercial segment of the Russian pharmaceutical segment.

Pharmstandard produces more than 200 medical products. Pharmstandard's product portfolio includes market-leading brands, such as Arbidol® (antiviral for systemic use), Pentalgin® (analgesics), Terpinod® (cough and cold), Complivit® (vitamins) and Flucostat® (antifungal). Key therapeutic segments for the Company are gene-engineering products (insulin, growth hormone), vitamins, and preparations for cardiology, immunology and gastroenterology.

Pharmstandard operates five manufacturing facilities in Russia and, with a production capacity of 1,350 million packs as of December 31, 2007, has one of the largest production capacities among domestic pharmaceutical companies in Russia. "Pharmstandard" follows GMP standard.

Pharmstandard generated sale of goods and profit of RUR11,371 million (US\$445 million) and RUR3,263 million (US\$128 million) million in 2007, respectively.



Группа компаний «Фармстандарт» основана в 2003 году. Управляющей компанией является ОАО «Фармстандарт», далее Компания.

Дочерние предприятия ОАО «Фармстандарт»:

Четыре фармацевтических завода:

- ОАО «Фармстандарт-Лексредства» (г.Курск),
- ОАО «Фармстандарт-Томскхимфарм» (г.Томск),
- ОАО «Фармстандарт-Уфавита» (г.Уфа),
- ООО «Фармстандарт-Фитофарм-НН» (г.Нижний Новгород).

Завод медицинской промышленности:

- ОАО «Тюменский завод медицинского оборудования и инструментов» (г.Тюмень).

На российском фармацевтическом рынке компания «Фармстандарт» является лидером среди отечественных производителей и входит в ТОП-3 среди всех фармацевтических компаний. Согласно данным ЦМИ «Фармэксперт» по итогам 2007 года «Фармстандарт» занял первую позицию в коммерческом сегменте рынка. Компания «Фармстандарт» выпускает более 200 наименований лекарственных средств. Лидирующими брендами Компании являются препараты «Арбидол» - препарат №1 по объему продаж в 2007 году, «Компливит», «Пенталгин», «Флюкостат» и «Коделак». Приоритетными направлениями развития - генно-инженерные, витаминно-минеральные, кардиологические, иммуномодулирующие и гастроэнтерологические препараты.

«Фармстандарт» владеет технологиями производства широкого спектра лекарственных форм: таблеток, растворимых таблеток, саше (растворимых порошков), сиропов, растворов, ампул, спреев, аэрозолей, желатиновых капсул, мазей.

Общий объем инвестиций ОАО «Фармстандарт» в модернизацию и развитие производства в 2004-2007 годах превысил 2,6 миллиарда рублей. В результате ввода в эксплуатацию новых производственных мощностей объем выпуска к концу 2007 года достиг 1 350 миллиона упаковок. В 2007 году объем выпуска готовых лекарственных средств превысил 600 миллионов упаковок. «Фармстандарт» соблюдает стандарт GMP при производстве лекарственных средств. Две линии на «Фармстандарт-Лексредства» (г.Курск) по производству твердых (таблеток) и жидких (сиропов) лекарственных форм подтвердили свое соответствие стандартам Европейского GMP.

В компании активно ведутся научные разработки новых высокотехнологичных препаратов в содружестве с ведущими научными учреждениями страны. На современном оборудовании «Фармстандарт-УфаВИТА» налажено производство генно-инженерных препаратов инсулина «Биосулины» и гормона роста человека «Растан».

Выручка «Фармстандарта» за 2007 год составила 11 371 миллион рублей, чистая прибыль – 3 263 миллиона рублей. Рыночная капитализация Компании составляет \$3,5 млрд.

«Фармстандарт» является лауреатом Национальной премии в области бизнеса «Компания года 2007» в номинации «Фармацевтическая промышленность».



ООО «Биоген-Аналитика»
Авторизованный дилер
BECKMAN COULTER Int.S.A.
РФ, 115093, Москва
Партийный пер., д.1, корп. 58, стр.1,
офис 34

Тел.: (495) 6609780
Факс: (495) 6609781
9331198@co.ru
<http://www.bga.su>

Компания ООО «Биоген-Аналитика» предоставляет широкий выбор аналитического и биотехнологического оборудования производства Beckman Coulter Int.S.A. (США):



- *рефрижераторные, ультра-, микро-, настольные, большегрузные, высокоскоростные центрифуги;*
- *жидкостные хроматографы;*
- *спектрофотометры;*
- *счетчики частиц и анализаторы пор;*
- *α - β -счетчики;*
- *pH-метры;*
- *капиллярный электрофорез;*
- *лабораторные роботы;*
- *системы генетического анализа;*
- *цитофлуориметры.*

Основными направлениям деятельности ООО "Биоген - Аналитика" являются:

- *продажа,*
- *поставка, инсталляция,*
- *гарантийное и послегарантийное обслуживание,*
- *а также методическая поддержка*

лабораторного, аналитического, биотехнологического и медицинского диагностического оборудования производства фирмы Beckman Coulter Int.S.A. (США) на территории России и стран СНГ.

Компания ООО «Биоген-Аналитика» имеет обученный персонал методистов и специалистов по продажам. Все сервисные инженеры проходят ежегодную переподготовку в центрах фирмы производителя Beckman Coulter Int.S.A. (США).

Название компании – ООО «Биоген-Аналитика»

Юридический и фактический адрес: 115093, Москва, ул. Партийный пер., д.1, корп. 58, стр. 1, оф.34

Тел.: (495) 660-97-80/81, 540-76 32

Факс: (495) 660-97-80/81, 540-76 32

E-mail: 9331198@co.ru, Grohmann@co.ru, ir@bga.su

Интернет: <http://www.bga.su>



Biogen-Analitica
Official representative of
BECKMAN COULTER Int.S.A.
115093, RF, Moscow,
Str. Partijniy per., 1, bld.58 – 1, of. 34

Tel.: (495) 6609780
Fax: (495) 6609781
9331198@co.ru
<http://www.bga.su>



“Biogen-Analitica” offers the wide variety of analytical and biotechnological equipment of production Beckman Coulter Int.S.A. (USA):

- **Centrifugation;**
- **HPLC;**
- **Spectrophotometers;**
- **Particle Characterisation;**
- **Scintillation Systems;**
- **pH Meters;**
- **Capillary Electrophoresis;**
- **Laboratory Automation Workstations;**
- **Genetic Analyses;**
- **Cytometry.**

The reference directions of activity of the company “Biogen-Analytica” » are

- Sale,
- Delivery and installation,
- Backup service and post-warranty service
- Methodical support

of the laboratorial, analytical, biotechnological and medical diagnostic equipment of production Beckman Coulter Int.S.A. (USA) firm in Russian Federation territory and the CIS countries.

The «Biogen-Analitica» company has the trained staff methodologists and sales managers. All service engineers pass yearly retraining in the Beckman Coulter Int.S.A. (USA) centers.

Biogen-Analitica Company

Address: 115093, Russian Federation, Moscow, Str. Partijniy per., 1, bld.58 – 1, of. 34

Tel.: (495) 660-97-80/81, 540-76 32

Fax: (495) 660-97-80/81, 540-76 32

E-mail: 9331198@co.ru, Grohmann@co.ru, ir@bga.su

Internet: <http://www.bga.su>



ЗАО "ХВД Биотех", HVD Biotech
117197, г. Москва, Ленинский пр-т 113/1, оф. Е-106;
Тел.: (495) 956-57-67; Факс: (495) 956-57-66,
E-mail: info@hvd.ru, www.hvd.ru

Два основных направления работы компании – это *InVitro* аллергодиагностика, Phadia АВ (Швеция) на приборах ImmunoCAP100 и ImmunoCAP250 и молекулярная биология.

В области молекулярной биологии, биотехнологии и геномных исследований мы предлагаем продукцию крупных производителей оборудования:

Pyrosequencing (Biotage АВ, Швеция) – автоматические системы для секвенирования ДНК в режиме реального времени. В основе метода лежит каскад ферментативных реакций, приводящий к появлению светового сигнала при включении каждого следующего нуклеотида в одноцепочечную ДНК-матрицу. Технология пиросеквенирования использует простые химические реакции и надежную систему детекции, исключающую необходимость применения гелей, радиоактивно меченных нуклеотидов и других специфических меток.

Biacore АВ (Швеция) – приборы для изучения взаимодействия биомолекул в режиме реального времени и комплектующие;

Мы предлагаем широкий выбор биочипов для изучения профилей генной экспрессии в различных организмах (человек, микроорганизмы, модельные организмы) компании Ocimum Biosolutions (Германия), а также специализированные тематические биочипы, отражающие гены, специфические при определенных патологиях: Синдром Дауна, онкологические заболевания, воспаление.

Мы предлагаем также градиентные ДНК-амплификаторы AutoQ компании Quanta Biotech (Англия) и автоматические системы для проведения капиллярного электрофореза HDA-GT12 производства компании eGene (США).



HVD Biotech
117197, Moscow, Leninsky Ave. 113/1, room E-106;
Tel.: (495) 956-57-67; Fax: (495) 956-57-66,
E-mail: info@hvd.ru, www.hvd.ru

HVD Biotech is an official distributor of following producers of equipment and services in field of molecular biology, biotechnology and genomic investigations:

Pyrosequencing (Biotage АВ) – automated systems for real time DNA sequencing and SNP-analysis;

Biacore АВ – real time label free biosensor analysis of biomolecular interactions;

Ocimum Biosolutions offers one of the largest selections of catalog arrays. This includes most comprehensive human arrays and covers most model organisms and many microorganisms.

systems for automated capillary electrophoresis eGene;

DNA-thermal cyclers Quanta Biotech.

Компания НТ-МДТ была основана в 1989 году. Первый образец продукции – сканирующий туннельный микроскоп был создан в 1990 году. Он до сих пор работает в Институте кристаллографии РАН. С момента основания и по сей день основное направление деятельности – создание научного оборудования для исследований во всех областях нанотехнологий.

В 1995 году был основан модельный ряд Solver (сканирующие зондовые микроскопы широкого профиля) и выпущена на рынок первая модель этого ряда. К настоящему времени модельный ряд насчитывает 11 моделей, которые установлены более чем в 700 лабораториях России, США, Японии, Западной Европы и других стран.

В 2003 был выпущен первый прибор для образовательных нужд – NanoEducator.

В 2004 была завершена разработка новой платформы – Интегра (зондовые нанолаборатории). На базе Интегра к настоящему времени выпущено 12 моделей, этот модельный ряд постоянно расширяется.

В 2006 году одна из базовых систем платформы – ИНТЕГРА СПЕКТРА получила приз американского журнала Research and Development, как лучшая инновационная разработка года в ряду приборов для научных исследований.

В 2006 году был введен в эксплуатацию первый сверхвысоковакуумный нанотехнологический комплекс НаноФаб. Запуск еще одного модельного ряда ознаменовал собой открытие нового направления в разработке научного оборудования, не только для компании НТ-МДТ, но и для всей мировой наноиндустрии — последние образцы этой продукции не имеют аналогов.

Генеральный директор Быков Виктор Александрович, доктор технических наук, лауреат премии правительства Российской Федерации в области науки и техники за 2005 год. Численность сотрудников головного предприятия более 240 человек. Компания тесно работает с аспирантурами МФТИ и МИЭТ.



Moscow, Zelenograd 124482, 317,
Box 158
Tel.: (495) 535-03-05
Fax: (495) 535-64-10
E-mail: spm@ntmbt.ru

NT-MDT has been creating the equipment for nanotechnology researches for more than 15 years, steadily holding advanced positions in the quality standards, high-tech developments and original solutions. The range of products is constantly expanding, and is represented by different equipment lines. Those are accessories for probe microscopy; SPMs for educational needs (NanoEducator); specialized SPMs (Solver) for scientific, industrial research centers. NT-MDT also produce probe nanolaboratories (NTEGRA) providing a wide spectrum of modern techniques on the SPM basis, and modular nanofactories (Nanofab) uniting the whole arsenal of means and techniques necessary for processing and quality control of devices and elements of micro- and nanoelectronics.

NT-MDT Co. was established in 1989 with the purpose to apply all acquired experience and knowledge in the field of nanotechnology to provide researchers with instruments suitable for solving any possible nanoscale tasks. The company was founded in Zelenograd — the center of Russian microelectronics.

The first scanning probe microscope for a wide range of investigations, Solver, appeared in 1995 starting Solver product line. By now, the Solver line formed by 11 different models for experiments in high vacuum, with biological matters, etc. More than 700 Solver devices have been installed in many countries worldwide: Japan, USA, Russia as well as asian and european countries.

In the year 2003 the first device for the educational purposes — NanoEducator — was produced.

The design of the new platform NTEGRA was finished in 2004. Currently company offers eight different systems based on the NTEGRA platform. They are dedicated to all the main applications and correspond to the newest trends in SPM. Each of these systems has its own specialization and serve as the superior instrument in its field. System for carrying out Raman spectroscopy, NTEGRA Spectra, won the R&D100 prize in the year 2006 as the best innovation system for scientific researches.

The first ultra-high-vacuum nanotechnological facility NanoFab was launched in 2006 marking with its unique features a new branch in the world nanoindustry.

The company has a wide distribution net and sales its products in 48 countries. The products development is based on the combination of MEMS technology, power of modern software, use of high-end microelectronic components and precision mechanical parts.

МАСС-СПЕКТРОМЕТРЫ APPLIEDBIOSYSTEMS/MDSSCIEX

Компания Applied Biosystems предлагает Вашему вниманию новую линейку масс-спектрометров для решения широкого круга задач аналитической химии. Предлагаемая линейка содержит как квадрупольные, так и времяпролетные масс-спектрометры. Большой выбор способов ионизации таких, как электроспрей, химическая ионизация при атмосферном давлении или фотоионизация, позволяет удовлетворить аналитические потребности любого исследователя. Представленные инструменты могут быть совмещены с жидкостными хроматографами различных производителей или работать с прямым вводом пробы.

Благодаря применению на всех приборах линейки запатентованных технологий камеры столкновений LINAC и «газовой завесы» стало возможным анализировать фемтограммовые количества веществ, понизить уровень шума и увеличить чувствительность.

Технология QTRAP позволяет сочетать все возможные режимы сканирования тройного квадрупольного с чувствительностью ионной ловушки. Инструмент QTRAP может быть использован как тройной квадрупольный масс-спектрометр для количественного анализа, но при этом существует возможность переключения одного из квадрупольных в режим работы «линейной ионной ловушки» - что дает возможность поиска и качественного анализа неизвестных веществ в пробе.



API 3200/3200 QTRAP

Масс-спектрометры серии API 3200/3200 QTRAP являются идеальным инструментом анализа практически всех типов веществ и концентраций в областях экологии, контроля качества продуктов питания, клинических исследованиях и фармакологии.

Диапазон масс: 5-1800 а.е.м.

Динамический диапазон: $1-4 \cdot 10^6$

Чувствительность на уровне пикограмм

API 4000/4000 QTRAP

Приборы серии API 4000/4000 QTRAP позволяют проводить как качественный, так и количественный анализ веществ в медицинских исследованиях и разработки новых лекарственных препаратов.

Диапазон масс: 5-3000 а.е.м.

Динамический диапазон: $1-4 \cdot 10^6$

Чувствительность на уровне фемтограмм



Для решения сложных протеомных задач компания Applied Biosystems представляет времяпролетные и комбинированные масс-спектрометры серий QSTAR Elite и 4800 MALDI. Данные системы позволяют проводить сложные информационно-зависимые эксперименты. Специально разработанное программное обеспечение делает возможным автоматическую смену режимов сканирования в зависимости от полученных данных. Наличие запатентованных систем выбора родительских ионов обеспечивает непревзойденную скорость и точность при переключении режимов анализа.

Ионно-оптическая система источника ионов с ортогональным облучением образца лазерным излучением позволили улучшить масс-спектрометрический анализ и увеличить чувствительность масс-спектрометра в 10 раз.

QSTAR Elite



Масс-спектрометр QSTAR Elite является комбинированным инструментом с квадрупольным и времяпролетным анализатором. Главным достоинством инструмента является высокая чувствительность и производительность анализа молекул в широчайшем диапазоне масс.

Диапазон масс: 5-40 000 а.е.м.

Разрешение >15000

Точность определения массы лучше 2 ppm

4800 MALDI TOF/TOF Plus

Непревзойденная чувствительность масс-спектрометра 4800 TOF/TOF делают его наиболее подходящим прибором для проведения сложных протеомных исследований. Ортогональное облучение образца лазерным излучением обеспечивает высокую чувствительность анализа. Высокая селективность выбора родительского иона в режимах MS/MS позволяет автоматизировать процесс идентификации белков и пептидов.



LC MALDI spotting



Система нанесения элюэнта на планшеты LC MALDI spotting позволяет в автоматическом режиме подготавливать матрицы для последующего анализа на масс-спектрометре. Система LC MALDI обеспечивает нанесение элюэнта со скоростью до 4 Гц за счет электростатического нанесения капли - без движения иглы в вертикальной плоскости.

Тел.: (495) 651 6797 Факс: (495) 651 6799

www.appliedbiosystems.com



119991, Moscow, Leninsky avenue, 47
Tel.: (499) 135-8802, 135-8818, 135-8823,
Fax: (495) 956-2701
E-mail: info@acrus.ru, sales@acrus.ru
www.acrus.ru

ACRUS company has already been in the Russian market of chemical reagents and laboratory equipment for more than 10 years. Our company has been performing as a reliable supplier of high quality European and American products to Russia.

ACRUS offers wide range of chemical reagents and equipment:

- **Imported reagents**
- **BULK products**
- **Biochemical reagents**
- **Pharmaceutical standards and impurity**
- **Analytical standards**
- **Solvents**
- **Magnitude nano- and micro-particles**
- **Spare parts**
- **Glass and plastic utensils**
- **Analytical instruments**
- **Laboratory furniture and equipment**



We can provide a complete procurement of R&D laboratories; biochemical laboratories; the laboratories working in the field of immunology; cell biology; genetic engineering; General QC laboratories; QC laboratories of oil refining and perfumery industries.

We deliver European and American catalogue reagents: *Acros Organics, Fisher BioReagents, Sigma-Aldrich, Chemicell, Prospec, MP Biomedicals, Invitrogen, Gibco, Merck, Alfa-Aesar (Avocado, Alfa-Aesar, Lancaster), Abcr, Amersham, Wenk LabTec GmbH, IsoLab, Whatman, Perkin Elmer, Kern, Sanyo Sartorius, Buchi, IKA, Eppendorf, Hyclone, Greiner Bi-One and others.* Our qualified personnel will help you to find any catalogue product You need.

Over 10,000 imported high quality reagent, instruments and equipment are available at stock in Acrus Moscow ware-house.

You can also order items You need to be delivered within 10 days after order placement.

We will be glad to welcome you among as regular clients!



✉ 119991, г. Москва, Ленинский проспект, д. 47
☎ (499) 135-8802, 135-8818, 135-8823,
Факс (495) 956-2701
e-mail: info@acrus.ru, sales@acrus.ru
www.acrus.ru

Компания АCRУС на рынке химических реактивов и оборудования в России уже более 10 лет. Наша компания зарекомендовала себя как надежный поставщик европейских и американских производителей.

АCRУС предлагает широкую линейку химической продукции и оборудования:

- **Импортные реактивы**
- **BULK – реактивы в оптовом количестве**
- **Биохимические реактивы**
- **Фармакопейные стандарты и их примеси**
- **Аналитические стандарты**
- **Растворители**
- **Магнитные нано- и микрочастицы**
- **Расходные материалы**
- **Стеклоянная и пластиковая посуда**
- **Аналитические приборы**
- **Лабораторное оборудование**
- **Мебель**



Мы можем обеспечить полную комплектацию научно-исследовательских лабораторий; биохимических лабораторий; лабораторий, работающих в области иммунологии, клеточной биологии, геной инженерии; лабораторий контроля качества; лабораторий нефтеперерабатывающей и парфюмерной промышленности; медицинских организаций; СЭС и др.

Мы осуществляем поставки по каталогам европейских и американских производителей: *Acros Organics, Fisher BioReagents, Sigma-Aldrich, Chemicell, Prospec, MP Biomedicals, Invitrogen, Gibco, Merck, Alfa-Aesar (Avocado, Alfa-Aesar, Lancaster), Abcr, Amersham, Wenk LabTec GmbH, IsoLab, Whatman, Perkin Elmer, Kern, Sanyo Sartorius, Buchi, IKA, Eppendorf, Hyclone, Greiner Bi-One* и др. Наши квалифицированные менеджеры помогут Вам подобрать интересующую продукцию по любому каталогу.

Компания Акрус имеет постоянно в наличии на складе в Москве около 10 тысяч импортных реактивов, расходных материалов и оборудования.

В случае отсутствия позиции на складе срок поставки – от 10 дней.

**Будем рады видеть Вас
среди постоянных клиентов нашей компании!**

При создании сборника использовались материалы Интернет-сайтов:

<http://www.toplab.de>
<http://www.cs.siue.edu>
http://btm.gujarat.gov.in/bio_informatics/genomics.html
<http://www.etseq.urv.es>
<http://pubs.acs.org>
<http://www.brynmawr.edu>
<http://istc.ru>
www.sgeier.net/fractals/indexe.php