for genomic regions based on nucleotide substitution frequency matrices, read depth and variant frequencies. We applied the heterogeneity estimation procedure to the genomes of pathogenic and opportunistic pathogenic bacteria newly sequenced by us, including 6 genomes of *Klebsiella pneumoniae,* 6 genomes of *Acinetobacter baumannii*, 4 genomes of *Mycoplasma hominis*, 4 genomes of *M. tuberculosis* and 3 genomes of *Staphylococcus aureus*. As a result, we found that the genomes of *M. hominis* are extremely heterogeneous (more than 4 times comparing to *K. pneumoniae*), while *M. tuberculosis* and *S. aureus* are slightly less heterogeneous, and *A. baumannii* and *K. pneumoniae* have substantially homogenous genomes. We plan to extensively study the available pathogenic genomes to re-assess their sequencing quality, reconstruct minor clonal variants based on NGS data, and get insights regarding the origins of their variability.

P.23-007-Mon
Virtual high-throughput screening for identification of *Plasmodium falciparum* protein kinase CK2-alpha inhibitors through targeting the active, allosteric and subunit interface sites

O. Mutlu
Marmara University, Istanbul, Turkey

Malaria remains the deadliest tropical disease caused by protozoan blood parasite *Plasmodium falciparum* and responsible from 216 million cases and half million deaths in 2016 despite many efforts. Clearly, identification of novel drug targets and drugs are urgently needed. Protein kinase CK2 (formerly casein kinase II) is an unspecific protein kinase which has more than 300 substrates to phosphorylate and have role in a wide range of cellular processes including cell cycle progression, survival, differentiation and proliferation. Recent studies show that the plasmodial CK2's one catalytic and two regulatory subunits are essential for the asexual blood stages of parasites and also having role in chromatin dynamics. In this study, we have focused on the protein kinase CK2-alpha subunit of *Plasmodium falciparum* (*Pf*CK2) to identify novel small chemicals and coumarin derivatives as drug candidates by virtual high-throughput screening workflow. Before screening, structural dynamics of the *Pf*CK2 was assessed by 25 ns long molecular dynamics simulations by using NAMD program. Analysing the structural dynamic nature of the protein, three different conformational structures were selected for the further analysis. Close to ten thousand small chemicals and coumarin derivatives from the ZINC and the PubChem databases were screened against one active site, three allosteric and one subunit interface sites of *Pf*CK2-alpha by Schrödinger Glide program. Based on the highest docking scores, outstanding results come from coumarin derivatives docked into the active site, allosteric site 3 that is related with the activation loop and closed conformation of the subunit interface. Taken together, novel inhibitor candidates targeting the allosteric site and subunit interface of highly druggable protein *Pf*CK2 were determined and selected for the further *in vitro* analyses by integrated virtual approaches.

P.23-008-Tue
Evolutionary conserved residues in proteins – looking beyond active site and structural core

M. Bzówka[1,2], T. Magdziarz[1], K. Mitusińska[1,2], A. Stańczak[1,2], A. Góra[1]
[1]*Tunneling Group, Biotechnology Centre, Silesian University of Technology, Gliwice, Poland,* [2]*Faculty of Chemistry, Silesian University of Technology, Gliwice, Poland*

Functionally important compartments of enzymes tends to be preserved during evolution of protein families. Active site residues are one of the most well-known examples. Due to their indisputable role in catalysis process, most of mutations results in unworkable proteins. Recent research shows that residues located on protein surface evolve faster than residues building protein core. Despite active site residues, there is no research concerning on the evolution rates of functionally important residues buried inside enzyme core. Growing evidence of the importance of transportation pathways linking the active site with protein surface raises the question of tunnels conservation. Problem is not trivial since tunnels may be equipped in gates and anchoring residues which contribute to selectivity of enzymes. This leads to opposing statements: i) due to critical role of tunnels residues building access pathways, tunnels might be conserved and ii) residues fine-tuning access to active site might be highly variable. In our research we are proposing a methodological approach for evolution rate analysis of any particular fragments of protein. As a testing system we have chosen epoxide hydrolases – enzymes with buried active site. Using combination of CAVER and molecular dynamics simulations we have identified several potential tunnels connecting active site with surrounding environment. Such long procedure was compared with simplified one based on crystal structure analysis only, which if accurate enough might be widely used in future for similar studies. The R package BALCONY (Better ALignment CONsensus analYsis) was used to facilitate entropy analysis of selected amino acids and comparison of evolutionary rates with other compartments of enzymes. Finally we are providing information about linking the evolution rates of particular residues with their functional analysis which gives insight into the strategy of enzyme adaptation and allows to identify functional hot spots.

P.23-009-Wed
Longevity in euarchontoglires: lost genes as a determinant

A. Seliverstov[1], L. Rubanov[1], G. Shilovsky[1,2], O. Zverkov[1], **V. Lyubetsky**[1,2]
[1]*Institute for Information Transmission Problems of the Russian Academy of Sciences (Kharkevich Institute), Moscow, Russia,* [2]*Lomonosov Moscow State University, Moscow, Russia*

In the 42nd FEBS Congress, we have reported the hypothesis that the loss of genes has notable evolutionary consequences; it has been exactly realized for specified potential genes in an original bioinformatics method based on the definition of a significant synteny disruption (http://lab6.iitp.ru/en/lossgainrsl). The method is applicable to data describing the absence or, vice versa, the presence of ontogenetic characters specific for these consequences. The method generates relatively short lists of genes for various data sets. First, we analyzed the body appendages regeneration and the telencephalon and eye development, and a diverse experimental testing of a gene identified by our method in *Xenopus laevis* called *c-Answer* coupled with the team of Prof. A. Zaraisky (Institute of Bioorganic Chemistry, Moscow, Russia) has confirmed the hypothesis (in press). Then the method was applied to the data on the genetic propensity of certain species for longevity and anti-

FEBS Open Bio **8** (Suppl. S1) (2018) 107–496 DOI: 10.1002/2211-5463.12453

ageing. The species with this ontogenetic property include mammals with a greater lifespan than could be expected from their body size: mole rats and primates (including the naked mole rat, Damaraland mole rat, capuchin monkeys, gibbons, western gorilla, bonobo, chimpanzee, human). The list of lost mouse genes includes *Smpd5, Spint5, Ttc41, Wap,* and *2310003L06Rik* expressed in the tongue, and certain vomeronasal and olfactory receptor genes. Overall, the revealed mouse genes demonstrate specific expression in reproduction-associated tissues, which agrees with the Williams' hypothesis concerning the reallocation of physiological recourses. The loss of some revealed vomeronasal and olfactory receptor genes in human and naked mole rat conforms to their special anatomical features. For this data, we suggest that the loss of certain genes in evolution is a determinant of lifespan elongation and ageing deceleration including neoteny. The research was supported by the Russian Science Foundation, pr.14-50-00150.

P.23-010-Mon
Bioinformatics analysis of a transgenic personalized murine model of refractory epilepsy

V. Sukhorukov[1], V. Kalmykov[1], A. Sharkov[1,2], P. Kusov[1,3]
[1]*Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia,* [2]*Research and Clinical Institute for Pediatrics at the Pirogov Russian National Research Medical University, Moscow, Russia,* [3]*Skolkovo Institute of Science and Technology, Moscow, Russia*

GRIN2A is a protein coding gene, which encoded NR2A subunit of NMDAR. A mutation in the exon 14 of the human GRIN2A gene was found in the patient (chr16: 9857449; G>G/A) and was associated to refractory epilepsy. In this study, we applied a bioinformatics approach to model murine mutated GRIN2A protein and appreciate possibility to create a murine model of this disease. The BLAST was used to find homologous mutation in murine GRIN2A gene. X-ray resolved structural models of the NR2A segments were used as source for M4T Raptor algorithm to generate predicted structure of mutated protein. The position of homologous point mutation in murine GRIN2A gene was revealed by the BLAST. Sequence alignment of the murine and human NR2A had 98% similarity, allowing to operate X-ray resolved structural models of the NR2A segments. It was shown that the replacement of Val440 to Ile440 amino acid via single nucleotide mutation, was located in S1 domain (404–539) region, forming loop called "loop 1" functioning as the NR1 NR2 ABD–ATD junction and heterodimer structural interface. Both residues were aliphatic and it was claimed, that this particular site is conservative mutation site. Nevertheless, observed changes in mutated protein model involve beta strands 2- and 3-elongated regions of beta sheets could be part of the structural compensation due to steric effect of bulkier than natural valine isoleucine residue. It is possible to assume, that NR2A disfunction can possibly lead to NMDAR hypofunction and neuron excitatory properties changes, causing higher nervous functions malfunction and further severe symptoms. It was shown that homologous mutation in human and murine GRIN2A gene led to the same conformational change in the protein structure and formed the mutated NR2A subunit. Thus, it is possible to create murine personalized model of the human refractory epilepsy. This study was supported by Russian Science Foundation (Grant #17-75-20249).

P.23-011-Tue
Application of a generic platform for the discovery and optimization of bio-active molecules

G. Nicolaes[1], K. Wichapong[1], B. Zarzycka[1], G. Vriend[2], T. Seijkens[3], T. Hackeng[1], C. Weber[4], W. Mulder[3], O. Soehnlein[4], E. Lutgens[3]
[1]*Cardiovascular Research Institute Maastricht, Maastricht, Netherlands,* [2]*RadboudUMC, Nijmegen, Netherlands,* [3]*Academic Medical Center, Amsterdam, Netherlands,* [4]*Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-University, Munich, Germany*

In today's molecular medicine, information exchange between experimental and computational approaches is vital for the rational study of clinically relevant proteins. The structural knowledge of such proteins provides the rational context that not only leads to a more profound understanding of the activities of such proteins, but also allows exploitation of such knowledge to provide novel means of diagnosis or therapy. We have built a generic platform that combines experimental approaches (i.e. basic biochemistry, molecular biology, peptide chemistry, surface plasmon resonance and ITC) with structural bioinformatics approaches (docking, molecular modeling, virtual ligand screening, molecular dynamics simulations and free energy calculations). With this platform, a team of experimentalists and bioinformaticians jointly address common research questions and adhere to a structure-driven approach to guide and rationalize functional work. This allows the identification and optimization of biologically active molecules that are active in vivo and that may be used as novel therapeutics or form the basis of novel modes of diagnosis. We have successfully identified small molecules and peptides against a variety of protein targets, both intra- and extracellular, including some from the fields of thrombosis, atherosclerosis, inflammation and antibiotic resistance. The variety of targets illustrates the synergistic power of our approach and moreover, we have shown that it is possible to obtain highly active molecules that express their activities also in vivo, via a cost-effective optimized non-traditional route of discovery and optimization.

P.23-012-Wed
The reliability of loop rebuilt by homology modelling in context of protein functionality and active site accessibility

K. Mitusińska, A. Góra
Biotechnology Centre Silesian University of Technology, Gliwice, Poland

The reliability of study based on protein structures strongly depends on quality of models and flexibility of particular compartments of macromolecules. Flexible and solvent-exposed regions like loops are prone to be poorly solved what hinders further protein analysis. Homology modelling is one of the widely used approach for reconstruction of missing residues in incomplete structures. The quality of obtained model is determined mostly by sequence similarity between used templates and size of the missing fragment, however scoring functions assessing accuracy of obtained models were not designed for flexible and solvent-exposed fragments of proteins. The structure of *Aspergillus niger* epoxide hydrolase is a good example: each crystal structure deposited at PDB database (Protein Data Bank) is missing a nine-amino-acids-long loop: 320TASAPNGAT328. This missing loop is located at the protein surface adjacent to the entrance of a buried active site in proximity of the catalytic pocket and thus