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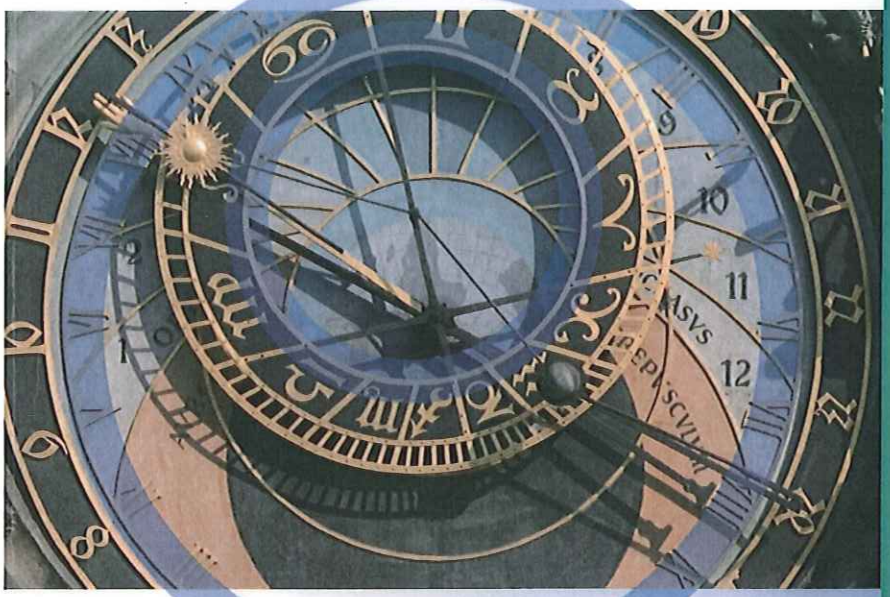

Life's Molecular Interactions

- Cells' Modular Components
- Social Life of the Cell
- Organism, the Network of Interactions

Czech Society for Biochemistry and Molecular Biology

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Abstracts

34th FEBS Congress

Life's Molecular Interactions

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plasmids coding for the purified fusion proteins. Large-scale expression targeted the protein production into the cell media. The Avi-tag purification protocol was further optimized in order to increase the fusion protein yield and purity sufficiently for protein crystallization experiments. The influence of tag sequence on the biochemical features of purified proteins and the ability to cleave the tag off by TEV protease treatment were additionally analysed. In conclusion, we present (i) a novel one-step purification protocol for GCPII, an intensively studied pharmaceutical target in prostate cancer and numerous neuro pathological disorders, and (ii) apply the same purification protocol on the study of NAALADaseL, a GCPII homolog of an unknown physiological function.

P2–138
Protein modeling program, fams-ace2 in CASP8, using the local consensus and 3D-1D quality assessment

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In the CASP8, our fams-ace2 server participated in the 3D coordinate prediction category as a human expert group. We applied two different scoring functions for the fully automated prediction server of the protein modeling, fams-ace2: (i) the local consensus score; and (ii) the model quality score based on classification of the side-chain environment for each residue. The local consensus score was used as a filter to select the models which have locally similar structures comparing with the set of models. The model quality score by our program CIRCLE was then used for the final selection of the best model. The procedure of fams-ace2 can be summarized as the following four steps: (i) Obviously incorrect models which have serious physical clashes or broken main-chain structures were removed. (ii) The top 10% of server models were selected in the order of the local consensus score. (iii) All of the server models, selected in step (ii), were refined and rebuilt utilizing our homology modeling program FAMS. (iv) The top five structures were selected, according to a model quality evaluation based on CIRCLE score. The coefficients of SS score in the circle which do not use the consensus method were changed in the fams-ace2. The fams-ace2 is a fully automated server and does not require human intervention. The parameters of fams-ace2 were optimized by the data set of previous CASP7. We used the GDT_TS as the quality of model compared to native. When we applied optimized fams-ace2 to CASP7 targets, fams-ace2 obtained the best results over all server groups. Moreover, in Template Based Modeling Targets, fams-ace2 also achieved best results over all groups including human groups. Although the advantage of fams-ace2 over other servers is slightly smaller than the results applying for CASP7 (123 domains), the intended results are accomplished. This small difference between CASP7 and CASP8 might be caused by the change of the distribution of target difficulty and performance of servers. When we calculate GDT_TS of CASP8 models, we did not consider the domain regions. Therefore the results of some targets will be changed. The advantages of fams-ace2 are the fully automated process, the lower calculation costs due to the decrease of the modeled number in comparison with Fams-ace1, and a high accuracy similar to the top of human groups.

P2–139
Study of the interactions between 14-3-3 protein and DNA-binding domain of forkhead transcription factor FOXO4

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The forkhead family of transcription factors shares a highly conserved DNA-binding domain. The FOX-proteins play roles in physiological and pathological processes. The FOXO-class consists of FOXO1, FOXO3, FOXO4 and FOXO6.These play a central role in cell-cycle control, differentiation, metabolism control, stress response and apoptoss. Transcriptional activity of FOXO is regulated through insulin-phosphatidylinositol-3-kinase-AKT/protein-kinase-B (PI3K-AKT/PKB) signaling pathway. The AKT/PKB-mediated phosphorylation triggers phosphorylation of additional sites and induces FOXO binding to 14-3-3.This promotes nuclear export of the complex and inhibits reimport of FOXO by interfering with its nuclear localization signal (NLS). The role of 14-3-3 in regulation of FOXO-transcription factors is two fold. Firstly, the 14-3-3 binding FOXO inhibits the interaction with DNA and secondly prevent its nuclear reimport by masking its NLS. We used fluorescence spectroscopy techniques to investigate the mechanism of 14-3-3-dependent inhibition of FOXO4/DNA binding properties. We labeled four sites within fork head domain of FOXO4 with extrinsic fluorophore 1,5-IAEDANS and used time-resolved fluorescence spectroscopy to study interaction between FOXO4 and 14-3-3.We suggest that 14-3-3 physically interacts with tested regions of fork head domain thus mask the DNA-binding interface thus blocking the FOXO4 binding to DNA. In addition, time-resolved tryptophan fluorescence indicates no significant 14-3-3 binding-induced conformational change of FOXO4 fork head domain. Thus 14-3-3 functions as a 'molecular-hood' that covers DNA-binding interface of FOXO4 and blocks its interaction with DNA.

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P2–140
14-3-3γ regulates CtBP1-S/BARS-mediated fission of post-golgi carriers

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Membrane fission is required during formation of intracellular transport carriers, a central process in membrane trafficking. Some fission mechanisms involve dynamin, and we have shown a fundamental role for CtBP1-S/BARS (BARS) at the Golgi complex and in fluid-phase endocytosis, defining a novel dynamin-independent fissioning machinery. To identify BARS-interacting proteins and define their roles in BARS-induced fission, we used