Session I: Moonlighting proteins

Lectures

L1.1

Matrix metalloproteinases in synaptic plasticity and memory

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Keywords: synaptic plasticity; metalloproteinases; LTP

Long-term synaptic plasticity at chemical synapses is regulated by activity of several extracellular proteases. Herein, we addressed the functions of MMP-3 protease in excitatory and inhibitory synaptic transmission and plasticity. Using field potential recordings in mice slices, we have shown that knockout of MMP-3 impaired late-LTP in CA3-CA1 hippocampal projection ($p<0.01$). Moreover, lack of MMP-3 activity affected induction of LTP dependent on L-type calcium channels, but not NMDA receptors. By means of in situ zymography and immunofluorescence, we found that LTP induction was associated with increased MMP-3 expression and activity. Additionally, in hippocampal slices, we observed MMP-3 in apposition to vGAT positive inhibitory synapses. Therefore, we studied the role of MMP-3 in inhibitory synaptic transmission using single molecule tracking of GABAA receptors. We have found that 45 min treatment of neuronal cultures with active MMP-3 decreased the diffusion coefficient of membrane GABAA receptors ($p<0.01$). Future work is needed to elucidate the impact of MMP-3 on the plasticity of inhibitory synapses. Overall, our observations indicate that activation of the MMP-3 regulates excitatory LTP and membrane mobility of GABA receptors.

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L1.2

FBPase - a moonlighting enzyme

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Keywords: FBPase; moonlighting; mitochondria; nucleus; cell survival; apoptosis

Muscle fructose 1,6-bisphosphatase (FBPase) is a well-known, evolutionarily-conserved enzyme that regulates glyconeogenesis. For years it seemed that its role in the cell has been well established and it has no more secrets to reveal. The discovery of FBPase in nuclei of some cell types showed this view to be false. Recently, more roles of FBPase have emerged such as involvement in cell survival, apoptosis and protection from reactive oxygen species, which seem to be independent of its enzymatic activity. The precise mechanisms of FBPase function in these processes remain unknown and are the subject of our most recent research. The discovery of novel roles of FBPase suggests that our knowledge of any protein's function should never be considered complete and exhaustive.

Posters

P1.1

Design of zinc baits as a tool for seeking out metal-mediated protein-protein interactions

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Keywords: zinc proteins; molecular baits; protein-protein interactions

Zinc is the most widespread transition metal which serves as a catalytic, structural and regulatory cofactor in hundreds of identified proteins. Bioinformatic analysis has further shown that the human proteome consists of at least 3000 proteins that potentially bind Zn^{2+} [1]. Such computational predictions are based on sequence homology with known zinc proteins, although this analysis does not include transient and interprotein binding sites. One of the most extensively studied interprotein zinc binding site is a zinc hook domain from the Rad50 protein, which forms a very stable zinc-mediated homodimer within the Mre11 complex and functions to repair double strand DNA breaks [2]. Our previous study on the minimal zinc hook domain shows that this interprotein metal-mediated site is highly attractive for protein engineering applications [3], although generally there is a lack of established methodology for seeking and identifying zinc-assisted protein-protein interactions. In this work, we sought to prepare and optimize various molecular baits immobilized on a solid support. Our approach uses two baits, the first built with dicysteinyl peptides with varying-length intercysteine linkers (AA**C**$(X)_n$**C**AA) and the second built with dihistididyl (AA**H**$(X)_n$**H**AA) motifs. These baits differ in their Zn^{2+} affinity and selectivity and may act as partners for naturally-occurring protein motifs. Specific optimization efforts focused on maximizing efficiency in protein partner binding.

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P1.2

Some nucleoside- and/or nucleotide-metabolizing enzymes can catalyze more than one type of reaction

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Keywords: multifunctional proteins; Fhits; nucleotide-metabolizing enzymes

Studying the metabolism of nucleosides and nucleotides, the following enzymes were found to catalyze more than one reaction: Nucleoside phosphotransferase (EC 2.7.1.77), which catalyzes the transfer of a phosphate residue from 5'-NMPs to nucleosides, can also hydrolyze NMP to nucleoside and P_i, thus acting as 5'-nucleotidase (EC 3.1.3.5) [1]. Adenosylhomocysteine hydrolase (EC 3.3.1.1), which splits adenosylhomocysteine to adenosine and L-homocysteine, also converts adenosine to adenine and ribose by forming an enzyme:adenosine complex, thus behaving like adenosine hydrolase (EC 3.2.2.7) [2]. Yeast exopolyphosphatase (EC 3.6.1.11), which catalyzes removal of orthophosphate from long-chain polyphosphates, cleaves adenosine tetraphosphate to ATP and P_i, thus acting as nucleoside tetraphosphatase (EC 3.6.1.14) [3]. Fhit proteins, which exhibited the greatest catalytic variation, behave as dinucleoside triphosphate hydrolases (EC 3.6.1.29), nucleoside phosphoramidases (EC 3.9.1.-), adenylylsulfate sulfohydrolases (EC 3.6.2.1) [4] and adenylylsulfate□ ammonia adenylyltransferases (EC 2.7.7.51) [5]. Finally, HIT proteins such as *A. thaliana* Hint4 and *C. elegans* DcpS, can act as adenylylsulfate sulfohydrolases (EC 3.6.2.1) or ADP sulfurylases (EC2.7.7.5) [5].

This observation should inspire studies on the structural basis of the catalytic capacities exhibited by the aforementioned proteins.

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P1.3

Extremely high affinity of zinc hook domain of Rad50 protein towards Zn^{2+}, Cd^{2+}, and Hg^{2+}

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Keywords: zinc hook; Rad50; affinity for Zn, Cd, Hg

The Rad50 protein is a constituent of the evolutionarily conserved Mre11-Rad50(-Nbs1/Xrs2) complex, which is involved in the DNA damage response pathway. Rad50 consists of a globular ATPase domain, a coiled coil region, and central β-hairpin with a zinc binding motif (zinc hook) that forms the apex of the structure and acts as a homodimerization factor. The minimal 14 amino acid hook (Hk) sequence is AKGK**C**PV**C**GRELTD and forms a highly stable complex with zinc (pK_d 19.2). Our study demonstrates that toxic Cd^{2+} and Hg^{2+} metal ions form even more stable complexes with Hk. A set of potentiometric, spectropolarimetric, and absorption UV-Vis titrations were conducted in order to analyse the stoichiometry of these metal complexes as well as the affinity of Hk towards Cd^{2+} and Hg^{2+}. Potentiometric and spectroscopic data from examining Cd^{2+}-Hk interaction indicate the formation of CdH_xL_2 complexes, similar to Zn^{2+}-Hk complexes. However, studies of Hg^{2+}-Hk interactions demonstrate the formation of HgH_xL_2 and Hg_2H_xL complexes. We found that Cd^{2+} and Hg^{2+} ions tend to substitute Zn^{2+} in $Zn(Hk)_2$ complexes, which confirms their higher stability (pK_d 21 and 21.5, respectively) when compared to all known metalloprotein complexes.

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P1.4

The most common mistakes in Western blot analysis based on the example of FTO protein

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Keywords: Western blot; ghost bands; no specifically binding by antibodies; FTO protein

The Western blot is a widely used analytical method for detecting specific proteins in a sample of tissue homogenate or protein extract. In order to obtain reliable results it is necessary to exclude sources of potential false positive results, such as: (I) primary antibodies recognizing unspecific binding to other proteins; and (II) secondary antibodies binding not only to primary antibodies but also to different immunoglobulins present in a sample. To avoid these false positive results we can use a number of approaches. First, it is advisable to verify the sequence recognized by the primary antibody in bioinformatic databases to check if the sequence of our protein is also present in proteins. The use of at least two antibodies directed against one protein but raised with different peptides confirms their specificity. Third, to avoid ghost protein bands it is necessary to use the proper concentration of antibodies in an experiment and do not applied too long time of exposure. To resolve the problem of the presence of immunoglobulins in the sample, we recommend using protein G or A immobilized on agarose for the specific task of removing these immunoglobulins. Last but not least, performing a transfection of siRNA gives us the greatest certainty that our antibodies recognize the examined protein. All these approaches we have trained on connected with FTO (**F**a**T** mass and **O**besity) protein, one out of nine human members of AlkB dioxygenase superfamily.

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P1.5

The studies on interaction of dephosphorylated 2'-5'- triadenylates and its analogues with human calmodulin as measured microscale thermophoresis and circular dichroism spectroscopy

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Keywords: calmodulin; oligoadenylates; thermophoresis; circular dichroism; Ca(II)

Dephosphorylated 2'-5'- triadenylates, a group of compounds composed of three adenylates connected via phosphate residue, are produce by 2'-5'- oligoadenylate synthase during viral infection. The direct mechanism of action of triadenylates on metabolism still remains unclear. In previous studies the interaction of tri- and others 2'-5'-oligoadenylates with distinct proteins like calmodulin, S100 proteins, RNAse L or DNA Topoisomerase I has been demonstrated.

Calmodulin (CaM) is calcium-binding protein expressed in all type of cells, involved in different metabolic pathways. Recently, we obtained an initial experimental data concerning possible regulation of Ca(II) binding affinity to CaM with 2'-5'-adenylates. With this hypothesis, we perform more detail studies on the interaction between CaM and dephosphorylated 2'-5'-triadenylate and its analogues using microscale thermophoresis (MST) and circular dichroism spectroscopy (CD) techniques.

The cDNA of human calmodulin was expressed in baculovirus system and purified according to previously published protocol. The interactions of 2'-5'- triadenylate and its derivatives with CaM in apo- (Ca(II) free) and holo- (Ca(II) saturated) forms were analyzed using nM and uM concentrations at pH 7.5.

The experimental conditions used in our studies more or less correspond to the 2'-5'- adenylates concentration in human cells during virus infection. Obtained results reveal binding of 2'-5'- triadenylates to CaM on the pico- or nanomolar level which means that such process could be observe *in vivo*. Also, our results exhibit absence of a statistically significant difference between binding of triadenylates to apo- and holo- forms of CaM.

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P1.6

The effect of some sulfur compounds on the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) *in vitro*

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Keywords: glyceraldehyde-3-phosphate dehydrogenase; post-translational modifications; lipoic acid; nitroglycerin

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) plays a role not only in the glycolysis pathway, but also in transcriptional activation and apoptosis. Furthermore, GAPDH takes part in the pathogenesis of neurodegenerative diseases, thus it is a classical example of a moonlighting protein. GAPDH undergoes a number of oxidative post-translational modifications of –SH group, which suppress its activity. Lipoic acid (LA) is used for therapy of a variety diseases including diabetic polyneuropathy, heavy metal intoxication and liver diseases. Recent studies have shown that LA inhibits S-nitrosylation, what may protect proteins from nitrosative and oxidative stress.

The aim of the study was to investigate the effect of LA and its reduced form dihydrolipoic acid (DHLA) on the GAPDH activity *in vitro* (potential S-lipoylation). Moreover, we examined whether LA or DHLA is able to restore the activity of GAPDH after the oxidative modification.

The obtained results showed that in the presence of LA, the GAPDH activity was significantly inhibited. This suggests that the enzyme is modified by S-lipoylation. In contrast, the impact of DHLA on GAPDH appeared to be insignificant.

Analogous studies of GAPDH activity were also carried out in the presence of NO donors: nitroglycerin (GTN), S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP). DHLA was able to reactivate GTN-treated enzyme, but it was ineffective against the enzyme treated with GSNO or SNAP. This suggests a different mechanism of inhibition of GAPDH activity.

In view of multifuncionality of GAPDH, this type of research can open new perspectives in the search for alternative methods of treating diabetes, neurodegenerative diseases, or cancer.