Friday, March 14, Session V

L21

RECOMBINANT ANTIBODIES FOR *IN VIVO* APPLICATIONS

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Interactions between biomolecules constitute the core of biological functions. In protein–protein interactions, specificity plays a key role and many proteins have evolved into extraordinarily precise molecular machines. The multistep phosphorylation pathway of the cytokinin signaling in plants involves promiscuous protein-protein interactions, allowing the plasticity of the plant-cell response to environmental and developmental stimuli. We have developed a recombinant antibody which is able to bind to a specific protein from the phosphorylation pathway in vivo. This protein cannot be phosphorylated in the presence of the recombinant antibody and the signal is not delivered to the nucleus to commit the reaction of the plant. To accomplish a successful selection of the recombinant antibody, we have developed an in vivo selection method. This method allows targeted selection of recombinant antibodies with a specific activity. During the development of our method, we came to these conclusions. In vivo selection process is important, because the stability of the recombinant antibody in the reducing environment of the plant cell is difficult to predict. Additionally, the uncertainty of the correct form of the antigen produced by heterologous expression introduces another variable, which might result in the unsuccessful selection of a correct recombinant antibody.

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SELECTION AND CHARACTERIAZATION OF ANTICALINS SPECIFIC FOR HUMAN GLUTAMATE CARBOXYPEPTIDASE II

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Prostate cancer (PCa) is the most prevalent tumor disease in man worldwide and as such is the subject of an extensive biomedical research. Glutamate carboxypeptidase II (GCPII) is a metallopeptidase overexpressed on the surface of castrate-resistant prostate tumors and is therefore exploited as a biomarker for the PCa imaging and therapy. Currently, only antibody and small molecule-based agents are used clinically for imaging PCa, although with variable degrees of success. As a novel strategy we are developing Anticalin-based binders specifically targeting GCPII.

Anticalins belong to a new generation of artificial binding proteins and a part their sequence can be randomized to obtain binding surface functionally similar to CDR of antibodies. We have used a synthetic combinatorial DNA library together with the phage display methodology to select Anticalin clones specific for the extracellular part of GCPII with affinities between 10 and 50 nM. By the affinity maturation technique where error-prone PCR was exploited to insert random mutations into the best Anticalin variant, we improved the affinity of the lead Anticalin to 1 nM. Furthermore, by the FACS analysis we showed that the best Anticalin clone is able to specifically bind mammalian cells expressing GCPII. These data were confirmed by immunofluorescence confocal microscopy. The best Anticalins will be subjected to next rounds of affinity maturation, crystallography assisted design and aimed at in vivo experiments to validate their biomedical potential.

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ROLE OF EF-HAND MOTIF IN THE ACTIVATION OF NEUTRAL TREHALASE

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Trehalases hydrolyze the non-reducing disaccharide trehalose amassed by cells as a universal protectant and storage carbohydrate. Recently, it has been shown that the activity of neutral trehalase Nth1 from *Saccharomyces cerevisiae* is mediated by the 14-3-3 protein binding that modulates the structure of both the catalytic domain and the region containing the EF-hand like motif which role in the activation of Nth1 is unclear. In this work, the structure of the Nth1:14-3-3 complex and the importance of the EF-hand like motif were investigated using site-directed mutagenesis, hydrogen/deuterium exchange coupled to mass spectrometry, chemical cross-linking and small angle X-ray scattering (SAXS). The low resolution structural views of Nth1 alone and the Nth1:14-3-3 complex show that the 14-3-3 protein binding induces a significant structural rearrangement of the whole Nth1 molecule. The EF-hand like motif-containing region forms a separate domain that interacts with both the 14-3-3 protein and the catalytic trehalase domain. The structural integrity of the EF-hand like motif is essential for the 14-3-3 protein-mediated activation of Nth1 and calcium binding, although not required for the activation, facilitates this process by affecting its structure. Our data suggest that the EF-hand like motif-containing domain functions as the intermediary through which the 14-3-3 protein modulates the function of the catalytic domain of Nth1.

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CHARACTERIZATION OF INTERACTIONS BETWEEN PROTEIN KINASE ASK1 AND ITS BINDING PARTNERS

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Apoptosis signal-regulating kinase 1 (ASK1), a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family, activates c-Jun N-terminal kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress, endoplasmic reticulum stress, and calcium ion influx. ASK1 plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration and cardiovascular diseases, thus being a promising therapeutic target against these pathologies. Enzymatic activity of ASK1 is tightly regulated by phosphorylation, oligomerization and protein-protein interactions. Formation of high molecular complexes, ASK1 signalosomes, was observed as an essential element for oxidative stress-induced cell death.

The 14-3-3 protein was identified as one of the most important physiological regulators of ASK1. It binds to the phosphorylated Ser967 at the C-terminus of the kinase domain ASK1 and maintains its inactive state, thus preventing the signaling initiation. It has been previously shown that ASK1 is activated after dephosphorylation of Ser967 and dissociation of 14-3-3 in the presence of ROS. Thioredoxine (TRX) binds to the N-terminal domain of ASK1 and it also prevents its activation. If exposed to ROS, TRX dissociates from ASK1 through unknown mechanism. This leads to the subsequent binding of TRAF2/6 and full activation. However, the precise mechanisms of processes that lead to ASK1 activation are still unclear. We present here the first structural and biophysical characterization of interactions between 14-3-3, TRX and corresponding binding domains of ASK1 using AUC, SAXS, time-resolved tryptophan fluorescence and mass spectrometry.

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CYTOMEGALOVIRUS GPUL141 EXHIBITS UNIQUE RECEPTOR-BINDING DOMAIN TO INHIBIT CELL SURFACE EXPRESSION OF CD155 AND TRAIL DEATH RECEPTORS

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To avoid immune recognition and to allow for longtime persistence in the host, human cytomegalovirus (HCMV) evolved a number of genes to evade or inhibit immune effector pathways. Especially glycoprotein (gp)UL141 can inhibit cell surface expression of both the natural killer (NK) cell activating ligand CD155 as well as TRAIL death receptors (TRAIL-R1 and -R2). The crystal structure of unliganded HCMV UL141 refined to 3.25 Å resolution allowed us to analyze its head-to-tail dimerization interface. We further designed a 'dimerization deficient' mutant of UL141 (ddUL141), which retained the ability to bind to TRAIL-R2 or CD155, while having lost the ability to cross-link two receptor monomers. Structural comparison of unliganded UL141 with UL141 that is bound to TRAIL-R2 further identified a mobile loop that makes intimate contacts with TRAIL-R2 upon receptor engagement. Superposition of the Ig-domain of UL141 on the CD155 ligand T-cell-Ig-and-ITIM-domain (TIGIT) revealed that UL141 can potentially engage CD155 similar to TIGIT by using the C'C" and GF loop. Further mutations in the TIGIT binding site of CD155 (Q63R and F128R) abrogated UL141 binding suggesting that the Ig domain of UL141 is a viral mimic of TIGIT, as it targets the same binding site on CD155 using similar 'lock-and-key' interactions. Sequence alignment of UL141 gene and its orthologs also showed conservation in this highly hydrophobic (L/A)X_6G 'lock' motif for CD155 binding as well as conservation along TRAIL-R2 binding patches, suggesting these host receptor interactions are evolutionary conserved.