ation rates with applied voltage demonstrates translocation of peptide. We further investigate the effect of modification by pegylation on peptide partition properties through the TomCC channel.

P33-032
Analysis of antiproliferative and antimetastatic effects of nNav 1.5 sodium channel and Notch-4 receptor inhibition

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Voltage gated sodium channel activity enhances cell behaviours related to metastasis, such as motility, invasion, oncogene expression. Neonatal alternative splice form of Nav1.5 isoform is expressed in metastatic breast cancers. Furthermore, aberrant Notch signalling can induce oncogenesis and may promote the progression of breast cancers. The aim of this research is the effect of the inhibition of these two molecules on the proliferation and metastatic behaviour of highly metastatic MDA-MB-231 human breast cancer cell as well as the interaction between these molecular systems. For this purpose, sodium channels were inhibited by an anticonvulsive drug phenytoin and the Notch-4 receptor signalling was inhibited by gamma secretase inhibitör, DAPT. In order to determine the individual and combined effects of these inhibitors, 4-[3-(4- iyodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3- benzene disulfonate (WST-1) test for proliferation, wound healing assay for lateral motility and zymography for **matrix metalloproteinase-9 (**MMP9) activity were performed. Finally, we found that the combined effect of DAPT and phenytoin is not as beneficial as using DAPT alone for decreasing metastatic properties of MDA-MB-231 breast cancer cells.

P33-034
VDAC activity in the presence of huntingtin proteins

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Huntington's disease (HD) is a progressive brain disorder that gradually robs affected individuals of memory, cognitive skills and normal movements. It is caused by the mutation of the gene encoding the huntingtin protein (Htt) that results in an increase of glutamine codon number above 35 and consequently in Htt with an abnormal stretch of above 35 glutamines in the N terminus (mHtt). At present it is becoming increasingly apparent that mHtt can impair mitochondrial function directly by affecting mitochondrial bioenergetics and dynamics. Thus, mitochondrial functioning appears to be affected by mHtt and the resulting mitochondrial impairments may occur early enough to contribute to mHtt-induced toxicity and HD pathogenic mechanism. Interestingly, the proposed mitochondrial targets of mHtt include processes that are known to be affected directly or indirectly by VDAC (voltage-dependent anion selective channel) located in the outer membrane of mitochondria and presently regarded as a global regulator, or governor, of mitochondrial functions. On the other hand, it is known that Htt interacts with above 200 proteins which represent a diverse array of biological functions. However, the functional relationship of Htt to mitochondria is still uncertain. Here we present our results concerning interactions between both Htt and mHtt and reconstituted human VDAC isoforms.

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P33-035
Regulation of serotonin transporter activity in animal models of peripheral inflammation – relevance to inflammation-induced depression

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Depression is a complex disorder precipitated in susceptible individuals by various stress factors. Inflammation is thought to be one such stressor, and chronic inflammatory diseases are often associated with depression. Molecular mechanisms underlying inflammation-induced depression are poorly understood. One target for immune system modulation of neuronal function is the serotonin transporter (SERT), a key regulator of serotonergic neurotransmission and a primary antidepressant target. Employing the widely used lipopolysaccharide (LPS) model of sickness and depression-like behaviour, we show that SERT activity is upregulated in the hippocampus and cortex of rats 24 h after LPS injection. The increase in SERT activity is not caused by enhanced mRNA or protein expression but due to posttranslational modification of the transporter. To complement the rather acute LPS model, we established a more clinically relevant model of chronic inflammatory diseases, namely the collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis. In the CIA model SERT activity is elevated in the hippocampus but not in other brain regions. Moreover, other neurochemical changes in CIA mice were found to correlate with previously observed effects in widely used chronic stress models of depression. For example, we found that BDNF mRNA levels are reduced in the CIA animals, suggesting that this model is suitable to study molecular mechanisms underlying depression triggered by chronic peripheral inflammation. Furthermore, comparative analysis of the two animal models allows us to characterise the neurochemical and behavioural consequences of acute and chronic peripheral immune system activation, including the modulation of serotonergic neurotransmission.

P33-036
The sea anemone *Heteractis crispa* – a source of potential pharmacological agents

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Venomous *marine organisms* are a unique source of compounds acting on various biological targets involved in important physiological processes. So, sea anemones produce a huge variety of

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neuro- and pore-forming polypeptide toxins, protease inhibitors, which can find wide applications in pharmacology. Such promising polypeptides are serine protease inhibitors, which, thanks to amino acid mutation at reactive site P1 position (Arg→Lys→Thr) during evolution have acquired the ability to interact with cysteine, aspartic proteases, and modulate Transient Receptor Potential (TRP) receptors and thus exhibit an analgesic effect *in vivo*.

We investigated structure-function relationships of two family representatives, so-called HC**GS**- and HC**RG**-polypeptides of Kunitz-type (with N-terminal GS and RG residues, respectively, each family of 33 polypeptides having point substitutions), which form *H. crispa* combinatorial library. Several polypeptides were obtained in the native state (In IV, InhVJ, HCRG 1, HCRG 2) or in recombinant form (HCGS 1.10, HCGS 1.36, HCRG 21). In contrast to other HCRG-polypeptides and similar to analgesic ones belonging to the HCGS family (Isaeva *et al*., 2012), HCRG 21 is characterized by Thr at P1 position. Polypeptides HCGS 1.10, HCGS 1.36, HCRG 21 demonstrated an analgesic effect *in vivo*. Electrophysiological assay of HCRG 21 on the TRPV1 receptor revealed 50% inhibitory activity (IC50 = ±10 μM). Molecular modeling (docking, mutagenesis, MD simulation) disclosed the functional significance of reactive site residues (at positions P1, 16, 17), and residues at 1, 5, 38 positions for polypeptides interacting with both biological target types.

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P33-037
Comparative analysis of Mg- dependent and Mg- independent HCO_3^-ATPases

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The comparative analysis between two enzymes, Mg-dependent and Mg-independent HCO_3-ATPases, were studied in synaptosomal and microsomal membrane fractions of albino rat brain, using the method of kinetic analysis of the multi-sited enzyme systems. Therefore, it can be inferred that Mg-dependent HCO_3-ATPase belongs to the group of "P type" transporting ATPases. Mg-independent HCO_3-ATPase with its kinetic properties may be attributed to the group of "Ecto" ATPases.

P33-038
Novel nitrate/nitrite transporter in the *Mycobacterium gilvum* Spyr1

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Microbial degradation is the major route by which Polycyclic Aromatic Hydrocarbons (PAHs) can be removed from the environment. Microbial activity is stimulated by addition of nutrients such as nitrate, which can serve as an electron acceptor under oxygen limitation conditions in contaminated soils. Nitrate is transported across the bacterial membrane by nitrate/nitrite porter proteins (NNP). Thus far, little is known about NNP genes in PAH-degrading bacteria. Genome sequencing [1] of the PAH-degrading bacterium *Mycobacterium gilvum* Spyr1 [2] revealed the existence of two putative NNP genes: *pynar* and *pynir*. Predicted gene products retain the characteristic nitrate-signature motifs (NS1 and NS2), conserved Gly and charged residues Arg within transmembrane segments 2 and 8 (R67, R268). NNP genes were cloned into an *E. coli* strain defective in all three endogenous nitrate/nitrite transporter genes (NarK, NarU and NirC). Heterologous expression of NNP genes was demonstrated by western blotting and net nitrate uptake assays were carried out. Our results indicate that *pynar* can complement the nitrate-dependent growth of the triple mutant and transport nitrate/nitrite in/out of bacteria. Mutants replacing R67 or R268 with Lys, His or Ala were found to be devoid of nitrate/nitrite transport activity.

[1] *Stand. Genomic Sci., 5:144 (2011)*
[2] *Appl. Biochem. Biotechnol.,159:155* (2009)

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P33-039
Characterization of ATP/ADP transporters (NTT) from obligate, intracellular living bacteria

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Adenine nucleotides are the major energy carriers in the cell. As their synthesis is limited to selected locations, cells rely for their passage across membranes on transporters. In mitochondria the ADP/ATP counter-exchange is mediated by ADP/ATP carriers (AACs), while in certain organisms a second, distinct system exists. These nucleotide translocator proteins (NTT) are structurally and functionally different from the AAC proteins, to which they posses no sequence similarity. They import cyotosolic ATP in exchange for ADP and phosphate in an electroneutral fashion.

NTT proteins are found in plant plastids and in the obligate, intracellular living orders of *Rickettsiales* and *Chlamydiales*, which rely on nucleotide import from the host for survival. They are also important pathogens (Epidemic typhus, Porcine proliferative Enteritis) continuing to kill more than 200.000 persons per year. The NTT proteins are absent from vertebrates, making them interesting drug targets, for which presently no inhibitors exist. At the moment the bacterial and plant NTT proteins have been, to different degrees, biochemically characterized, yet no detailed structural information is available.

The NTT proteins from a range of different bacteria could be successfully over expressed, purified and further characterized, with crystallization experiments being underway. Effects of detergent and buffer conditions and stability were assayed using a GFP tag protein. In addition the effects of mutants, targeting basic residues or testing truncations, in the plant AtNTT1 protein point to functionally relevant loop regions and residues of the protein, which can be exploited for crystallization and give further insights into the function.

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