**P4-066**  
**DISRUPTED NUCLEAR TRANSPORT: A CENTRAL EVENT IN ALZHEIMER’S PATHOPHYSIOLOGY**  
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**Background:** Transcription of DNA is regulated by the actions of several classes of proteins on nuclear DNA. Epigenetic molecules are central to this process. **Methods:** We used immunohistochemistry, in situ hybridization, Western blotting, siRNA and in vitro methods in human, t.g. mice and cell lines to examine cellular localization and expression of epigenetic molecules. **Results:** Alzheimer’s disease results in inability of transcription regulatory molecules to access neuronal nuclei, with an accompanying build up of these molecules in the cytoplasm. These effects involve altered expression of RAN and may be driven by specific forms of Abeta. **Conclusions:** Our data suggest a model in which failed localization of transcription regulatory molecules to the cell nucleus can have dramatic effects on transcription.

**P4-067**  
**CANNABINOID LIGANDS DIRECTLY MODIFY BETΑ-AMYLOID FIBRIL FORMATION AND ARE VARIBLY NEUROPROTECTIVE IN VITRO**  
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**Background:** Cannabinoid (CB) ligands such as anandamide and cannabidiol afford neuroprotection against β-amyloid exposure. Their mechanism of protection is not clear, particularly with respect to pharmacological selectivity. The possibility that cannabinoid ligands may directly interfere with β-amyloid formation has yet to be investigated, even though some share similar moieties to known polyphenols capable of disrupting β-amyloid. In this study we determined the capacity for a set of structurally and pharmacologically diverse cannabinoid ligands to directly alter amyloid fibril formation and compared this with their neuroprotective properties in vitro. **Methods:** Cannabinoid ligands were assessed for direct interaction with Ab 1-42 fibril formation via a fluorometric Thioflavin T (ThT) binding assay. In addition, transmission electron microscopy (TEM) was used to directly visualise the effects of CB ligands on Ab 1-42 fibril aggregation and morphology, using the following compounds: anandamide and 2-arachidonoyl glycerol (2-AG) (endoogenous CB ligands), ACEA (CB1 receptor-selective agonist), JWH-015 (CB2 receptor-selective agonist), cannabidiol (CBD: phytocannabinoid), Abnormal-cannabidiol (Abn-CBD), O-1602 and O-1918 (putative atypical CB/GPR55 receptor ligands). In addition, human neuroblastoma SH-SYSY cells were treated with β-amyloid (Ab 1-42; 0.1-5 μM) for 24 hours, alone or in the presence of select CB ligands based on results of the ThT and TEM screening. **Results:** ThT assay revealed maximal Ab 1-42 fibril formation at 6 hrs with continued aggregation over a further 8 hrs. Extensive and significant overall inhibition of ThT fluorescence (45-75%) occurred from incubation of Ab 1-42 with the following CB ligands over this period (Abn-CBD > > THC = O-1602 > CBD > 2-AG). TEM of fibrilll imaging however was discordant with the ThT results, with only O-1602 altering Ab 1-42 structure to an amorphous state versus control fibrils. O-1918 and 2-AG also altered fibrill morpholoy to an amorphous appearance, without effect on ThT fluorescence. SH-SYSY cells exhibited reduced cell viability following exposure to Ab 1-42, with only ACEA inhibiting Ab 1-42-mediated neurotoxicity. **Conclusions:** Cannabinoid ligand effects on ThT fluorescence alone are not predictive of an anti-amyloid, neuroprotective potential. However, some cannabinoid ligands were found to alter fibrilll morphology and the neuroprotective correlates to this effect merit further investigation. Selected cannabinoid agonists targeting CB1 receptors augment neuronal protection to β-amyloid.

**P4-068**  
**MODIFICATIONS TO UREA SDS AB-PAGE FACILITATE THE RESOLUTION OF N-TERMINALLY TRUNCATED AB PEPTIDES AND OF AB FORMS LONGER THAN 42 RESIDUES**  
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**Background:** The Bicinic/ bis-tris/ tris sulphate PAGE system (Wiltfang J et al., Electrophoresis 12, 352-366, 1991) with 8 M urea in the separation gel (Klaffki HW et al., Anal Biochem 237, 24-29, 1996) is a well established method for the electrophoretic separation of different C-terminal Ab variants (1-37 to 1-42). In addition to Ab peptides starting at asparagine 1, N-terminally truncated Ab peptides may be of particular pathophysiological relevance (Seergeat N et al., J Neurochem 85, 1581-1591, 2003). The analysis of long Ab peptides, such as 1-46 to 1-49 is particularly interesting in the context of the mechanism of γ-secretase cleavage (Niu X, J Alzheimers Dis 16, 211-224, 2009). Here we describe the optimisation of the gel matrix to reach baseline separation of these additional Ab species. **Methods:** To optimise the separation gel matrix, pH, urea, acrylamide, and SDS concentration were varied. Separation performance was assessed using either synthetic Ab peptides or supernatants from SH-SYSY cells. Ab peptides were detected on western blots using mAbs 1E8 and 6E10. **Results:** Improved separation of N-terminal Ab variants starting at aspartic acid (1), alanine (2) glutamic acid (3), or pyrogulactam acid (3) was achieved by modification of the H 2 SO 4 concentration and the pore size of the separation gel. In addition, observations regarding “long” Ab peptides, such as 1-43, 1-44, 1-45, 1-46, 1-47, 1-48, and 1-49 will be presented. **Conclusions:** Modifications of the buffer conditions in urea SDS AB-PAGE allow the resolution of selected Ab variants, customised to a specific biological question. Increased resolution helps to analyze complex Ab patterns, as for example in cell culture supernatants, but also in other biological samples.

**P4-069**  
**IMAGING OF BETΑ-AMYLOID METAL BINDING IN YEAST WITH X-RAY FLUORESCENCE MICROSCOPY**  
Victor Streltsov¹, Simon James², Martin de Jonge², Daryl Howard², David Paterson³, Sonia Sankovich¹, Jo Caine¹, ¹CSIRO, Melbourne, Australia; ²Australian Synchrotron, Melbourne, Australia.  
**Background:** Alzheimer’s disease (AD) is the most common form of dementia in humans and is related to the accumulation of the amyloid-β peptide (Ab). Ab contains a high affinity (Cu, Zn and Fe) metal binding site that along with modulating peptide aggregation can also generate toxic reactive oxygen species (ROS). Details of metal distribution in cells and the mode of Ab-metal binding may be critical to the etiology of AD. Caine et al (FEMS Yeast Res. 2007, 7, 1230-1236) indicated that yeast may be a tractable model system for the screening metals re-distribution and toxicity caused by Ab. The intracellular Ab was produced through fusion with a green fluorescent protein (GFP) in yeast. The Ab fusions promote stress in yeast cells suggesting that intracellular Ab is toxic. The GFP-Ab fluorescence localises into high density punctate cytoplasmic patches. Here we use x-ray fluorescence microscopy (XFM) to map metals in model yeast system expressing GFP-Ab. **Methods:** The distribution of trace elements in Saccharomyces cerevisiae yeast (BY4743) cells was measured with Zone Plate (ZP) nanorobe and Vortex silicon drift detector at the Australian synchrotron XFM beamline. Ab-1-42 was cloned onto the C-terminus of the GFP within the expression vector pAS1N-GFP and pAS1N-GFP was used as the control. Addition of metal solutions was also monitored by flow cytometry. **Results:** XFM results (Figure) indicate that GFP-Ab expressing cells exposed to Cu ions accumulated larger levels of copper compared to levels of zinc. In addition, flow cytometry showed accelerated aggregation of the GFP-Ab in the presence of metals. This is consistent with studies by Suazo et al (Biochim.Bioph ys.Res.Commun. 2009, 382, 740-744) which showed that human HEK293 cells overexpressing APP and human APP751 transformed into the FRE1 ferric reductase defective S. cerevisiae yeast cells increased Cu uptake when exposed to Cu ions. Moreover, wild-type HEK293 cells exposed to both Cu ions and APP135-155 synthetic peptides also increased copper uptake. **Conclusions:** Using XFM nanorobe we find that expression of Ab in yeast model cells facilitates copper uptake and may represent a step in cellular copper homeostasis. These results emphasize the importance of trace biometals in AD-linked Ab-mediated protein aggregation in cells.
**Conclusions:** APP increases an output of Ab via the interaction among Abeta, p75NTR and dent manner, and the increased interaction of p75NTR-APP may participate phins enhance the interaction of p75NTR-APP in a phosphorylation depen-
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**Background:** Amyloid beta (Ab), originating from cleavage of APP (amy-
lloid precursor protein), is a hallmark of Alzheimer’s disease. P75NTR is known to regulate Abeta deposition in the brain but its role in APP trafficking and processing is not known. **Methods:** We use FRET (fluorescence resonance energy transfer) analysis to characterize the nature of the APP-p75NTR interaction in HEK293 cells which were transfected with p75NTR-CFP and APP-YFP plasmids and controls. These cells were subjected to a range of neurotrophins, and Ab, at differing concentrations. Co-immunoprecipitation and fractionation were used to examine the interaction between p75NTR and APP. **Results:** The FRET efficiency of APP-p75NTR was significantly higher than the negative control. All neuro-
trophins triggered an increase in the FRET efficiency between p75NTR and APP. Interestingly, Ab at all concentrations triggered a dramatic increase in FRET efficiency. The interaction increased dramatically within the first 1-3 minutes of the addition of Ab to the cells. Co-IP data suggest that Abeta en-
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cAMP was added, FRET efficiency increased significantly. This data seems to support the idea that p75NTR-APP interaction is under the influence of a phosphorylation event. Furthermore FRET efficiency dropped further by blocking endogenous Ab with an antibody 6E10, indicating endogenous Ab may increase the interaction of APP and p75NTR. These results could suggest that Ab may have a positive feed forward mechanism that increases an output of Ab via the interaction among Abeta, p75NTR and APP. **Conclusions:** We conclude that p75NTR ligands Ab and neurotrophins enhance the interaction of p75NTR-APP in a phosphorylation depend-

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