

**P006** Production, purification and characterisation of recombinant human proNGF and binding to the TrkA, p75<sup>NTR</sup> and Sortilin receptors

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The neurotrophins are a family of proteins that provide trophic support to neurons of the central and peripheral nervous system. They mediate their actions through a common p75<sup>NTR</sup> neurotrophin receptor, as well as specific tyrosine kinase receptors (Trks). Nerve growth factor (NGF), the prototypic neurotrophin, binds to TrkA. Cholinergic neurons of the basal forebrain are particularly vulnerable in Alzheimer's disease and loss of these neurons early in the disease is associated with memory loss and cognitive impairment. The cholinergic cells are unique in the brain in that they express both TrkA and p75<sup>NTR</sup>. Recently it was shown that when proNGF binds to p75<sup>NTR</sup>, together with the receptor sortilin, apoptosis may be initiated. ProNGF has been shown to be increased in Alzheimer brain, leading to speculation that this may be implicated in cholinergic neuron loss.

In order to fully characterise the binding kinetics of proNGF to its receptors we have produced recombinant human proNGF, wild-type, without tags or extraneous amino acids present. In addition we have produced a mutated form of proNGF, resistant to proteolytic cleavage. We have characterised the binding of these proteins to sortilin, p75<sup>NTR</sup> and TrkA in HEK cells expressing the receptors and by surface plasmon resonance.