

P003 Neurotensin Receptor 1: *E.coli* expression, purification, characterisation and biophysical studies

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Neurotensin (NT) is an endogenous tridecapeptide neurotransmitter found in the central nervous system and gastrointestinal tract. One receptor for NT, NTS-1, belongs to the G protein coupled receptor (GPCR) superfamily, has seven putative transmembrane (TM) domains, and is being studied by a range of single molecule, functional and structural approaches.

To enable biophysical characterisation, sufficient quantities of the receptor need to be expressed and purified in active form.

To this end, rat NTS1 has been expressed in *E.coli*, in an active, ligand-binding form at the cell membrane. Active receptor has then been produced in sufficient amounts for structural biology studies either with or without fluorescent protein (YFP and CFP) fusions. Ligand-binding is demonstrated in a novel SPR approach using tagged ligand and the receptor as the analyte [1], as well as by conventional radioligand binding and by fluorescence measurements.

Solid state NMR has been used to interrogate the NT binding site [3 - 6], and modelling of the protein and ligand have given indications of the mode of ligand-receptor interactions. Electron microscopy shows that the detergent cocktail devised, produces a monodisperse receptor, suitable for further studies.