

## How do untranslated regions of cannabinoid receptor type 1 mRNA determine receptor subcellular localisation and function

### Supervisory team:

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### Project description:

The endocannabinoid system (ECS) is a homeostatic mechanism that plays key roles in a wide range of brain functions including cognition, learning, appetite, and pain. One way it does this is by reducing the amount of neurotransmitter released at synapses to prevent excessive neuronal activity. This modulation of synaptic function is mediated by the very precise localisation of the primary endocannabinoid receptor, CB1R, at the presynaptic membrane. However, the ECS also has different, and sometimes opposing, functions mediated by CB1Rs located at different subcellular compartments, particularly at mitochondria. How these different pools of CB1R are established and maintained, and how their differential functions are controlled, is poorly understood.

CB1R transcription can generate mRNA isoforms with different untranslated regions (UTRs). UTR-mediated mechanisms are intimately involved in the regulation of mRNA trafficking and its subsequent local translation. For example, RNA binding protein (RBP) interactions with UTRs of BDNF mRNA determine activity-dependent translation at distinct cellular locations in response to specific stimuli. We hypothesise that analogous mechanisms control CB1R localisation and function.

The aim of this project is to explore how different UTR variants direct trafficking and translation of CB1R mRNAs to different cellular CB1R pools that serve different functions.

**Techniques:** The project will involve genetic manipulation of primary neurons to express and monitor the subcellular localisation and functions of CB1Rs with alternative UTRs. To achieve this the student will be trained in a wide variety of molecular, protein, and cell biology techniques, including *in silico* analysis; cloning; virus production; cell culture; Western blotting; co-immunoprecipitation; live and fixed cell confocal imaging; neurotransmitter release, electrophysiology etc in culture and *in vivo*.

**Objectives:** 1) Identify of novel CB1R-UTRs through *in silico* analysis and experimentation and map RNA-binding protein (RBP) sites in the alternative CB1R-UTRs. 2) Investigate the trafficking of CB1R mRNAs with alternative UTRs. 3) Define the effects of neuronal activity on alternative CB1R-UTR selection. 4) Determine how UTR mediated mechanisms drive CB1R to interact with distinct proteins, such as in homo/heterodimerization. 5) Understand the consequences of alternative CB1R-UTRs on ECS signalling.

**Outcomes:** This integrated multidisciplinary approach and combination of techniques will provide excellent PhD training. The results obtained will define the roles of CB1R UTRs and provide important new insights into how the ECS is regulated, and if these pathways could be manipulated for beneficial effect.