

## Poster Presentation

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### *Structural Studies of the DCC-Ribosome Complex*

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During neuronal development axons are guided by a gradient of the signal molecule netrin, which attracts extending neurons by binding to the DCC (Deleted in Colorectal Cancer) Receptor. It has been shown that the intracellular domain of the DCC receptor interacts directly with the ribosome, and that this interaction is crucial for axon guidance(1). However structural insights into this interaction are still lacking. Our aim is therefore to determine the crystal structure of the DCC-ribosome complex. As membrane proteins can be challenging to crystallize, especially together with a huge macromolecular complex as the ribosome, a cloned fragment of the intracellular domain of the human DCC receptor will be used. Tcherkezian et al. showed that the interaction with the ribosome occurs through the ribosomal protein L5(1), which is conserved from *S. cerevisiae* to humans. We therefore decided to use the 80S *S. cerevisiae* ribosome because crystallization conditions are known and well-established (2). Furthermore, in the crystals of *S. cerevisiae* there is a large solvent channel passing by ribosomal protein L5(2), making it likely that we can soak fragments of the DCC receptor into crystals of *S. cerevisiae* ribosome. In order to screen for soluble fragments of the DCC receptor that can be used for soaking, a random PCR expression approach first described by Kawasaki and Inagaki(3), where random PCR fragments are inserted into a vector containing GFP was used. This method resulted in several soluble fragments covering the P1 domain that was shown to be responsible for ribosome binding(1). These fragments are now being purified, and will be tested for ribosome binding, and subsequently in soaking and co-crystallisation experiments. Progress will be presented.

[1] Tcherkezian J, Brittis PA, Thomas F, Roux PP, & Flanagan JG (2010) Transmembrane receptor DCC associates with protein synthesis machinery and regulates translation. *Cell* 141(4):632-644., [2] Ben-Shem A, Jenner L, Yusupova G, & Yusupov M (2010) Crystal structure of the eukaryotic ribosome. *Science* 330(6008):1203-1209., [3] Kawasaki M & Inagaki F (2001) Random PCR-based screening for soluble domains using green fluorescent protein. *Biochemical and biophysical research communications* 280(3):842-844.

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