Characterizing Orphan GPCRs in Molluscan Neurons through Bioinformatics

**Introduction:** Orphan receptors, receptors that have been identified in a genome but whose function and endogenous ligand have not been identified, are a problem for neuroscientists due to the limited amount of knowledge regarding them. Many orphan receptors are G-protein coupled receptors (GPCRs), which are involved in a variety of biological processes. This project involves a novel method in studying orphan receptors by first locating them in the gene libraries of molluscan species, in particular gastropods commonly known as "sea slugs."

**Methods:** Fragmented RNA sequences from gastropod species are assembled into contiguous sequences and are collected into a transcriptome, a library of genes being expressed in the brain tissue. Bioinformatics tools provide information about genes in those libraries, including topological structure of proteins, expression levels, and homology. Genes that match the profile of orphan GPCRs can be identified and referenced using web-based gene databases. After orphan receptor candidates are located in the transcriptomes, their gene sequences are then used to isolate the orphan receptors in the cDNA of the mollusc. The gene is then cloned through a bacterial vector and used in in-situ hybridization of the whole animal brain in order to determine in which neurons the receptor is being expressed. By using previously acquired knowledge of the neuronal circuitry of these animals, a ligand may be identified through single-cell sequencing of presynaptic neurons or testing of responses to different molecules.

**Results:** Several orphan GPCR candidates have been identified in the transcriptome of *Tritonia diomedea*, including GPR-83 and GPR-139, which have been confirmed as having unknown ligands. The neuronal homes of these receptors’ expression are now being determined via in-situ hybridization.

**Conclusion:** The identification of orphan receptors in the gene sequences of invertebrate species represents a powerful technique for characterizing orphan receptors found in the human genome. Identifying ligands for these receptors is important for both other scientific projects that concern the receptors and for development of drugs targeted at tissue containing them.