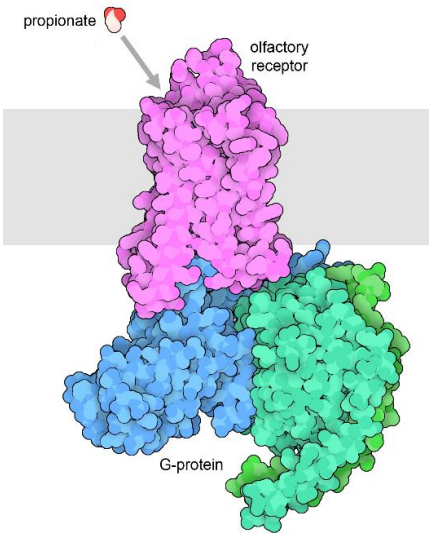
Proposal for Investigating the Mechanisms of Odor Detection in the OR2 Superfamily of GPCR Olfactory Receptors

Chemical sensing and odor perception is the oldest and primary chemical sense in aquatic and terrestrial organisms. This is reflected in the olfactory gene family, which is the largest, comprising up to 1% of all genes in mammals[1].

The sensing of odors is not well understood. In the last seventy-five years there have been attempts to classify odors from a biological/chemical perspective and map them to G protein-coupled olfactory receptors (GPCRs). The proteins responsible for olfactory receptor function constitute the largest family of membrane proteins in humans and other mammals. There are over 400 genes in the odor-sensing human genome and over a thousand in mice. These olfactory receptors (ORs) are expressed within olfactory sensory neurons, part of the olfactory neurons in the olfactory epithelium that extend via axons to the olfactory bulb of the brain[2–8]. These ORs can be highly specific or promiscuous in their reactions to the triggering molecules. Odor interpretation is not only based on triggering ORs but is also influenced by concentration. Cyclopentyl mercaptan and butyric acid in extremely low concentrations are pleasant food notes, interpreted by nasal ORs during eating, but in higher concentrations trigger a repulsive reaction by most humans[9].



The goal of this research is to expand the data we have on OR2 superfamily of GPCR olfactory receptor conformational changes when exposed to specific odor compounds and submit them into the M2OR database. This research will focus on ORs and compounds not currently researched and in the M2OR database for the OR2 superfamily. Currently, the physical determination of structural conformation can be challenging for large molecules; therefore, GPCR molecular conformations will be predicted computationally. If feasible, depending on the OR molecule, a physical structural analysis will be performed to confirm the predicted protein conformation. If the molecular pocket of the OR protein has ligand receptors reactive to multiple odor molecule types, multiple computation cycles will be run, one for each odor molecule-OR pair. Those that appear promising and conducive to physical examination will be analyzed via X-ray crystallography or cryo-electron microscopy (Cryo EM) for the base OR protein. With a low binding affinity (high K_D) between OR and odor molecules, physical analysis may not produce acceptable conformational structure data[10].

Figure 1 Olfactory receptor (magenta). G-proteins are shown in blue and green. The odorant propionate is shown at top. [From PDB-101 (Training and outreach programs of Protein Database), molecule of the month, first figure, https://pdb101.rcsb.org/motm/282.]

Candidates for research will be from the intersection of human and mouse OR proteins of the OR2 superfamily, without data already in the M2OR database. The intersection allows future research to include in vivo testing to determine whether our analyses of conformational changes are related to the levels of detection in living organisms. By limiting the proteins to the human/mouse intersection, the researchers will have some idea of how the mice will interpret the odor molecules, that is, fruity, pleasant, citrusy, et cetera. This study is restricted solely to the protein structure and structural changes in response to odor molecules. The plan is to map the gene through DNA/RNA to the protein and the conformational changes triggering the olfactory nerve for many unmapped GPCR olfactory receptor paths. If the research proceeds to in vivo trials, the data gathered will encompass more of this olfactory processing path. Under the assumption that the OR protein is amenable to physical analysis, DNA will need to be synthesized, incorporated into a plasmid, and inserted into an E. coli candidate to produce the OR protein. This will require collaboration with biologists with experience in this area.

The GPCRdb acts as a source of GPCR OR structures[11]. Structures that have not already been studied and are not in the M2OR database will be candidates for this study. Software tools such as gpcrTASSER and RoseTTAFold, in conjunction with AutoDock Vina, will be used to computationally predict structures and small-molecule interactions. Owing to the high L_Ds of the protein/target ligand complex, obtaining reliable data from physical analyses is challenging; however, relying on computational data alone bears its own risk.

After computational studies, a protein's properties dictate whether the protein is likely to yield successful data from a physical analysis. If so, it will be used to synthesize DNA. The synthesis will be performed using Enzymatic DNA Synthesis (EDS). When the base DNA is ready, it and a set of appropriate DNA primers will be used, using DNA PCR, to generate the required quantity of DNA. These will be incorporated into plasmids via restriction-based cloning. Once the plasmids are prepared, they will be incorporated into E. coli via bacterial transformation. E. coli can then be cultured to produce OR protein molecules. The E. coli will be lysed, and the OR protein will be purified via ion-exchange and size exclusion chromatography. Given the presence of cystine disulfide bridges in OR proteins, the SHuffle or Origami strains of E. coli will be used for protein biosynthesis. X-ray crystallization and/or Cryo EM will be used for conformational analysis.

Of significant interest is the interaction between the extracellular loop 2 (ECL2) of the OR proteins and its effect on protein-ligand conformation. Research indicates that ECL2 is a critical component in ligand bond formation and the odor detection pathway[12]. Additional structural analysis will include 3D-quantitative structure-activity relationship (3D-QSAR) studies to help correlate similarities in OR subgroups with similarities in odor molecule structure. This can lead to the prediction of molecules that interact with OR proteins. An important aspect of all results is to ensure that the data meets the M2OR data requirements.

Future research may include in vivo testing and protein mutation to determine how the protein conformational structure/ligand formation targets different molecules for sensing.

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